Evaluation of spatial variability of metal bioavailability in soils using geostatistics

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ABSTRACT BOOK
6th SETAC World Congress/SETAC Europe 22nd Annual Meeting

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This book composes the abstracts of the presentations for the platform and poster sessions of the 6th World Congress/22nd Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), conducted at the Estrel Hotel in Berlin, Germany from 20-24 May 2012.
The abstracts are reproduced as accepted by the scientific committee of the meeting and appear in order of abstract code, in alphabetical order per presentation type. The poster spotlight abstracts and poster corners abstracts are included in the list of poster abstracts.

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SOCIETY OF ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists, and others interested in environmental issues such as managers and engineers. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in 1979 to fill the void. Based on growing membership, meetings attendance, and publications, the forum was needed.

A unique strength of SETAC is its commitment to balance the interests of academia, business, and government. The Society by-laws mandate equal representation from these three sectors for World Council Officers, Board of Directors / Council Members, and Committee members. And although there is no control mechanism, the proportion of members from each of the three sectors has remained nearly equal over the past 30 years.

Like many other professional societies, SETAC publishes esteemed scientific journals and convenes annual meetings replete with state-of-the-science poster and platform presentations. Because of its multidisciplinary approach, however, the scope of the science of SETAC is much broader in concept and application than that of most other societies.

SETAC is concerned about global environmental issues. Its members are committed to good science worldwide, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur.

SETAC was founded in North America but membership was open to environmental scientists worldwide. SETAC Europe was organized in 1989; SETAC Asia / Pacific in 1997 and SETAC Latin America in 1999. Members voted overwhelmingly in 2001 to combine these "geographic units" into one global society to form the SETAC World Council. SETAC meets the professional needs of individuals at local and regional levels throughout all geographic units, throughout national branches and chapters (Argentina, Brazil, United Kingdom, Central and Eastern Europe, Africa and soon-to-be organized Japan), through regional chapters, and through national language branches (Germany). International acceptance of the SETAC model continues with widespread interest in Russia and Africa. It is now the job of SETAC World Council to oversee the myriad SETAC activities around the world and to assure the integrity of the Society.

Environmental Toxicology and Chemistry, an internationally acclaimed scientific journal, has grown from a quarterly publication of fewer than 400 pages annually in 1982 to a monthly publication of 2.915 pages in 2001. Since January 2005, SETAC publishes a second scientific journal: Integrated Environmental Assessment and Management. IEAM is devoted to bridge the gap between scientific research and the use of science in decision making, regulation and environmental management. SETAC publishes the global newsletter SETAC Globe, peer-reviewed workshop and symposia proceedings, and a variety of technical reports.

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Keynote speaker abstracts

KS01
The Biological Basis of Sustainability
Jeff McNeely
International Union for Conservation of Nature (IUCN)
Sustainability requires the capacity to adapt to changing conditions, and the ecosystems of our planet are the result of billions of years of successfully adapting to change. Their diversity provides today's societies with a wide range of services, ranging from provision of living resources to pollination and protection against natural hazards. In recent years, many indusries are learning from natural systems, finding that "biomimicry" can help to solve many challenges in efficient and sustainable product design -- everything from architecture that requires less energy to paint that washes itself. The "natural infrastructure" provided by nature continues to provide sustainable benefits to people, with no costs of contruction or maintenance. The genetic diversity of plants will be essential for adapting to changing climates and meeting the increasing demands for food by a growing human population. And what does nature need in return? Being treated with respect, some sacrifices in oppotunity costs, and being given full consideration as critical elements in the strategies that governments and the private sector are developing to support sustainable development. The environmental foundations of sustainability will be essential for building the the economic and social dimensions of future human wellbeing.

KS02
Sustainability – an ecological, economic and social programme for future development
Jochen Flasbarth
President of the Federal Environment Agency, Dessau-Roßlau, Germany
The concept of sustainable development was introduced to the political and scientific debate 20 years ago at the UNCED conference in Rio de Janeiro. Since then we have seen, on the one hand, rapid growth of economies worldwide, increased resource consumption and apparent evidence of climate change. On the other hand there is growing awareness of public society that planetary boundaries exist and that the limited resources should be shared with developing countries and economies in transition. Technical and political concepts have been developed for how to save energy and resources, avoid toxic emissions and tackle major problems like rising energy demand, loss of fertile soils, lack of drinking water, demographic development, etc. 20 years after 1992, governments and stakeholders will meet again in Rio. What have we achieved? What should we achieve? And how can chemistry contribute to sustainable development?
Despite all the progress already achieved we need a fundamental change in the economy and lifestyle if we want future generations to be able to meet their needs. If business continues as usual, according to estimates by UNEP, energy-related CO2 emissions will increase continuously to more than 50 gigatonnes by 2030, although we need a reversal of emission trends if we want to comply with the 2°C target. The global demand for water will exceed supplies by 40 percent as early as 2030. According to the World Water Council currently more than 60 percent - in arid regions even 90 percent - of water withdrawals is used for irrigation.
The world economy needs to be changed in such a way that the limits of the global ecosystem are respected and irreversible damage is avoided. That means that we urgently need a greening of the economy which respects the planetary boundaries by setting environmental objectives. Key fields of action in industrialized countries include the transition to a low-carbon economy, reducing resource consumption in absolute terms and preserving biodiversity. The transformation to a green economy is a precondition for sustainable development and requires the contribution of the private sector in particular. Green economy is not an illusion of environmentalists which can only be realized in ecological niches. To the contrary, green economy offers major opportunities for business and employment and relates to the entire spectrum of economic activities. Various studies show that e.g. ambitious climate protection goals can create additional jobs, for example in developing renewable energies and in renovating buildings to enhance their energy efficiency. Looking at the chemical sector it can also be demonstrated that eco-innovations offer win-win situations for companies active in that sector.
However, a green economy will not develop on its own. In order to accelerate the transition process towards a green economy we need economic incentives for eco-innovations and sustainable consumption and production patterns. In industrialized countries, environmentally harmful subsidies need to be reduced and economic actors who behave unsustainably should bear the social costs they cause. We need a transformation from short-term to long-term responsibility as societies.
The chemical sector is one of the key sectors of Europe's economy. It is highly innovative and can therefore provide solutions to achieve the goals of sustainable development. In the past pollution by chemical industry was nearly synonymous with environmental degradation in general. Significant progress has been achieved in the meantime but problems and deficiencies still remain to be solved. One of the commitments made in Johannesburg in 2002 was that "chemicals should be used and produced in ways that lead to the minimization of significant adverse effects on human health and the environment". In Europe, REACH may provide safe chemicals but not necessarily sustainable chemicals. Complying with environmental laws is not sufficient to meet the requirements of energy and resource efficiency as well as sustainable production. Petroleum is still the main feedstock for producing chemicals. The chemical industry is not sufficiently prepared to move towards renewable alternatives or – in the long-term - to hydrogen-based production.
The addressees of a sustainable chemicals policy do not only include the chemical industry. There must also be fundamental changes in agricultural practice in order to reduce the use of pesticides, veterinary pharmaceuticals and arable soil. Producing and using increasing amounts of chemicals, pesticides and pharmaceuticals is unsustainable in ecological, economic and social terms.

KS03
Resource Efficiency and Decoupling as Drivers for Sustainability
Mr. Arab Hoballah
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The remarkable economic and population growth of the 20th century was coupled to substantial increases in the extraction and consumption of natural resources, leading to increasingly-damaging negative environmental impacts. Total material extraction increased by a factor of about 8, and the price index of resources declined by about 30%. But many resources are now reaching their productive limits, as indicated by rising prices and lower grades of ores being mined.
However, economic growth globally was faster than growth of the rate of natural resources' consumption, and some negative environmental impacts have been reduced. Globally, about 25% less material input was required in 2002 compared to 1980 to produce one unit of real GDP which is a move towards a more resource efficient society. It appears that some 'dematerialization' of the world economy has occurred spontaneously.
Accelerating this process of decoupling economic activity from consumption and environmental impacts is fundamental to future human well-being. Sustainability demands that resources be used more efficiently reducing economic and environmental costs of resource depletion and negative environmental impacts. Finally, while in some parts of the world local environmental impacts have declined, global impacts on the environment are becoming ever more important and severe, requiring an international policy response. Future policies will require a better understanding of the process, so the International Resource Panel (IRP) has undertaken to define the key issues and challenges, drawing on peer-reviewed research and international experiences [1]. In seeking to find where policy interventions for decoupling will be most effective, it is essential to consider the life cycle of resources, from initial extraction to ultimate disposal (or recycling) [2]. A major conclusion drawn by the IRP [1] is that decoupling is feasible, and is already happening, but further sustainability-oriented innovations are urgently required to enable decoupling to support sustainable development more effectively. More transformative change is required to meet the size of the challenge.

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[2] UNEP (2004) Why Take a Life Cycle Approach. Paris

Special session abstracts

SS01-1
A Statement on the Sustainability of Global Human Societies: Toward a Declaration of the 6th SETAC World Congress, May 2012
L Kapustka
SRL Consulting, OTTAWA, Canada
As we develop the underpinnings for what will become the Berlin Declaration, we recognize the unique opportunity that SETAC has to contribute meaningfully to the global dialogue on sustainability. SETAC's tripartite structure of governmental, academic, and industry scientists and environmental professionals offers a model system for the trans-disciplinary development of a science of sustainability. Since its inception in 1979, SETAC's orientation has evolved from primarily environmental to include many aspects of social, economic and behavioural sciences, including decision analysis and policy analysis.
SETAC enters the worldwide sustainability discussion in an interesting time. A society's ability to reach into any corner of the planet and produce a needed material from the global resource base enables the development of megacities and in general allows our present society to live in a state far from equilibrium with our local landscapes. This reach also allows us to occupy marginal lands that could not support even the smallest of human communities long-term. Living on marginal lands and importing food, fibre, and energy places great demand from the goods side of the ecological landscape (the rate at which the system supplies desired food, water, and other materials), but also from the services side (the assimilation rate of societal wastes).
As these challenges are discussed, a new paradigm is emerging, one that is different from the widely endorsed three pillars of sustainability. The new paradigm considers a nested relationship in which economies reside within societies, which in turn are wholly dependent upon surrounding ecological systems. This new perspective relates to the Millennium Ecosystem Assessment that highlights societies' dependency on ecosystem services. SETAC's challenge is to embrace and reinforce this science-informed conceptual model of sustainable social - ecological landscapes, within this professional society and out into the greater sustainability community. The Statement is building toward a Declaration that will offer a view of how the emerging science of sustainability can guide and inform societal decision-making processes.

SS01-2
Integrated Sustainability Assessment: Providing Scientific Support for Sustainability Policy
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Sustainability is among the founding, long-term goals of the European Union and has been adopted as a guiding principle and objective for policy development by the European Commission. The EU Sustainable Development Strategy advances a vision for sustainable, prosperous communities predicated on resource efficiency, environmental protection and social cohesion. The Europe 2020 Strategy for 'Smart, Sustainable and Inclusive Growth' provides the policy agenda for sustainability.
The Sustainability Assessment Unit (Institute for Environment and Sustainability, DG Joint Research Centre) aims to advance science-based decision support for sustainability policy formulation, monitoring, and cross-cutting impact assessment. Towards this end, the Unit develops new methods and approaches for integrated sustainability assessment, including accounting frameworks and monitoring tools, reference data, and scenario modelling platforms to ultimately support policy making within the EU.
The activities of the Unit are more and more targeted towards the implementation of objectives and policies foreseen within the Europe 2020 Strategy. This includes flagship initiatives such as 'A Resource Efficient Europe', the 'Sustainable Consumption and Production and Sustainable Industrial Policy Action Plan', the Strategy for 'Innovating for Sustainable Growth: a Bioeconomy for Europe,' and the 'The Raw Materials Initiative - Meeting our Critical Needs for Growth and Jobs in Europe'. Seeking to integrate and expand upon these initiatives, on-going development combines life cycle environmental, social and economic analyses within a common framework where indicator results can be assessed against sustainability targets and thresholds, or be used within a (land use) modelling platform to support the assessment of development policies via regionally-resolved sustainability scenarios.
Life cycle thinking - one of the core elements of Unit activities - is implemented via the European Platform on LCA. In recognition of the pivotal importance of quality-assured data for robust assessments, the International Reference Life Cycle Data Network (ILCD) was created as a repository for sharing quality-compliant Life Cycle Inventory (emissions and resource use) data sets. It is open to all providers including business, national LCA projects, researchers, consultants, and others. Building on the ILCD, the Life Cycle-based Indicators and the European Environmental Footprint provide the methods and framework for life cycle assessments of environmental impacts related to European production and consumption.

SS01-3
Micronutrient to the rescue! How zinc fights a global health problem
E. Van Genderen
International Zinc Association, Durham, United States of America
Due to the current dependence on metals in society, the industry has focused its research programs on many attributes that contribute to the sustainable development of basic resources - food, water and energy. For essential metals, such as zinc, the linkage between soil and human deficiency has been identified as an area that requires significant attention in order to address childhood mortality through improved agricultural productivity. For example, zinc deficiency is attributed to 800,000 deaths per year (450,000 deaths per year in children under the age of five). Further, it has been estimated that 50% of the world's agricultural soils are deficient in zinc. This high prevalence of zinc deficient soils in major agricultural zones limits crop productivity and lowers the nutritional value. In light of these critical and linked issues of zinc deficiency in soils, crops and humans, the International Zinc Association has launched programs that promote both short-term (food supplementation initiatives in conjunction with UNICEF) and long-term (improved crop production and nutrition through use of zinc containing fertilizers) strategies to address this global health problem. The presentation will provide an overview of these activities, including education outreach, policy efforts, communications and research.

SS01-4
Sustaining the Potential - making the most of nanotechnology
A. Davies
Defra Chemicals and Nanotechnologies Division, London, United Kingdom
Nanomaterials form an emerging and extremely promising technology, which already offers a wide range of economic and societal benefits and has the potential to unlock some of 21st Century society's biggest challenges. There are already encouraging indications that nanoscience can be an enabler to the environmental agenda, as it is in health care, electronics, construction, information technology and many other areas. However, to unlock this potential, Governments will need to bring science and policy development together, so that the benefits are maximised and the risks minimised. Developing partnerships will be key, as will the take-up of concepts such as responsible care; but all this needs to rest on a sound scientific evidence base, including life cycle analysis and risk assessment. This paper will explore these themes from the perspective of the United Kingdom and the European Union.

SS01-5
Sustainability communications: the do's and don'ts
J.F. Fava
PE International & Five Winds Strategic Consulting, West chester, United States of America
Organizations are seeking ways to continuingly describe their improved social, environmental and financial performance. Enterprise wide and product specific guidelines have been developed by a variety of groups and governments, e.g., the Global Reporting Initiative (GRI), International Organization for Standardization (ISO), DJSI, FTC environmental claims guidance, and others. These efforts ensure a level playing field by advancing harmonized 'rules' on how an organization should communication their story/progress without 'green washing'. Additionally, with the increase in communication mechanisms, organizations are learning how to better utilize the internet and smart phones to reach broader audiences in new ways. This presentation will provide examples of good and 'not so good' sustainability communications and will conclude with general thoughts on what should be considered when developing and implementing a sustainability communications strategy.

SS02-1
Exposure assessment in ERA: from current tools to new approaches
A Di Guardo
University of Insubria, COMO, Italy
Environmental exposure can be assessed by means of monitoring activities and/or predictive approaches such as fate and bioaccumulation modelling. Current approaches in EU are mainly based on the use of models to estimate the fate of a chemical, as the Technical Guidance Document (TGD) dictates; however, these approaches, generally provide a steady state calculation of the fate of the chemical in a generic, standard "average"European scenario. Such static approach neglects temporal and spatial variability and can be currently adopted as a predictive tool for chemicals belonging to the classical "non polar"category, unless measured partition coefficients are provided. To overcome such issue, models should incorporate algorithm for predicting partitioning of e.g., polar and ionized chemicals, and account for more spatial and temporal variability, considering the diversity of ecosystem exposure conditions. On the monitoring side, a number of environmental data and experimental parameters are needed to improve the modelling approaches: from data on mixtures of chemical, (not commonly assessed as such), to the nanomaterials, for which a considerable lack of understanding of the fate still exists. Other knowledge gaps can be found in specific areas in which modelling approaches can be improved, from mass transfer parameters in specific environmental

scenarios to realistic environmental compartment characteristics and "validation"datasets, to name a few.

SS02-2
Environmental effect assessment and risk characterization of chemicals: what's wrong and how can do it better?
C. Janssen
Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent, Belgium
Ecological risk assessment (RA) procedures form the basis for the development of national and international regulations aimed at protecting the environment against potential adverse effects of chemical substances. The currently used approaches, however, lack environmental realism which leads to high uncertainty and use of largely unsubstantiated 'safety factors'. This paper will review some major drawbacks of current environmental/ecological effects assessment methods and propose new approaches based on recent scientific developments.

SS02-3
Mechanistic effect modelling for ecological risk assessment: state-of-the art, trends, and challenges
V Grimm
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Mechanistic effect modelling includes individual-level effect models, such as TK/TD and Dynamic Energy Budget models, and ecological models addressing populations and communities. Such models have the potential to overcome limitations of current risk assessment, in particular regarding higher tiers, complex exposure patterns, indirect ecological effects, and endpoints relevant for ecosystem vulnerability and services. The state-of-the art includes models that have been shown to be realistic enough to make robust predictions relevant for risk assessment of chemicals. However, so far such models had to be assessed on a case-by-case basis and often were not designed and communicated in a way that makes them suitable for regulatory decisions. Therefore, current initiatives are trying to establish guidelines for Good Modelling Practice, for example the TRACE documentation framework developed in the EU-funded project CREAM. Main current challenges include: establishing acceptance criteria for mechanistic effect models, which then become part of Good Modelling Practice; agreement on standard species, scenarios, models, and ecological endpoints; installation of procedures that prevent standard models from becoming static and therefore being used uncritically; and linking effect and exposure models. Mechanistic effects models are an indispensable tool for making risk assessment of chemicals more ecologically relevant. However, concerted actions, which correspond in scope and required effort to FOCUS, are needed to fully integrate such models in future regulatory risk assessments.

SS02-4
Addressing complexity in ecological systems - ignore it or embrace it?
V.E. Forbes
University of Nebraska-Lincoln, Lincoln, United States of America
Despite a widespread and long-held recognition that ecological systems are complex, traditional approaches to ecological risk assessment have largely attempted to ignore complexity. Instead, standard approaches rely on simplistic tests and assumptions with complexities glossed over by applying fixed 'safety' or 'uncertainty' factors. Advances in computer science, together with the development of appropriate modeling tools and modeling practice are facilitating more sophisticated and scientifically robust ways to deal with complexity. For example, extrapolation of effects across levels of biological organization, consideration of multiple stressors, and complex exposure scenarios can all be addressed using appropriate agent-based models (ABMs). ABMs offer many benefits for ecological risk assessment: they are highly flexible, not limited by mathematical tractability, can incorporate spatial and temporal variability, can capture interactions among species and among multiple stressors, and are ideal for studying responses that cross levels of biological organization. There remain challenges for both the modeling and for model implementation so that the necessary complexities can be effectively addressed. The alternative, of course, is to continue to keep the complexities implicit and hope that by doing so will make them go away.

SS02-5
Priorities to improve ecological risk assessment for chemicals
T.C.M. Brock
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The ecosystem services concept can be applied to operationalize and harmonize the generic protection goals formulated in legislative documents. For each ecosystem service potentially affected by chemicals, important taxa/functional groups need to be identified. It is key to identify "potential vulnerable"representatives (focal species) of these taxa/functional groups on which the different risk assessment tiers should focus. From a risk manger and cost-benifit point of view it may be necessary to develop criteria for a transparent spatial differentiation in specific protection goal options. Lack of a clear conceptual basis for the interface between the exposure and the effect assessment may lead to an overall low scientific quality of the risk assessment. This interface is defined as the Ecotoxicologically Relevant Concentration (ERC). Key is that the type of ERC used to express the "C"in the exposure estimates should not be in conflict with the ERC used to express the "C"in effect estimate. In the near future many of the ERC problems might be solved by using toxicokinetic / toxicodynamic or population models and exposure scenarios specifically developed for "vulnerable"focal species.The different tiers in ERA schemes need to be calibrated. An important lesson learned from the past is that the consistency of the tiered approach needs to be re-evaluated every time new chemistries (with a novel toxic mode-of-action) come on the market. An important pitfall in ERA remains the extrapolation of results of relatively simple model ecosystem experiments and computer simulation models to the diverse reality of the field. In relatively simple model ecosystem experiments and food-web models the indirect effects of chemical-stress observed may be a caricature of reality, since not all essential feedback mechanisms that may dampen temporal chemical-stress in natural ecosystems will be captured in the simple models.In the extended abstract more detailed information is given.

SS02-6
Regulatory point of view (focused on the implementation of REACH) on the new scientific challenges for ecological risk assessment on hazardous and PBT substances
L Vakra, J. V. Tarazona, B. Versonnen
European Chemicals Agency, HELSINKI, Finland
Disclaimer: The authors are staff members of the European Chemicals Agency. The views expressed are solely the authors' views and do not represent an official position of the Agency The presentation will focus on the regulatory point of view (focused on implementation of REACH) on the new scientific challenges for ecological risk assessment in the case of hazardous and PBT substances which, as part of the registration process, require an environmental exposure assessment (EEA), the environmental risk characterisation, and/or the identification of operational conditions (OC) and/or risk management measures (RMM) for minimizing the emissions to the environment. The presentation will cover the following topics:- different roles and responsibilities under REACH involving EEA and risk characterisation/emission minimisation;- substances registered that require an EEA and risk characterisation/emission minimisation; and the evaluation process;- REACH-related regulatory difficulties related to EEA and risk characterisation/emission minimisation;- scientific challenges in EEA and risk characterisation/emission minimisation faced during dossier evaluation so far;- major EEA and risk characterisation/emission minimisation related deficiencies in dossiers evaluated so far: current experience, further needs and possible solutions;- relevance of the identified challenges and proposed recommendations in the draft SCCS/SCHER/SCENIHR opinion regarding the implementation of the REACH Regulation;- further suggestions and research needs in the regulatory scientific context.

SS02-7
The view of a regulator on the possibilities for applying more ecologically relevant effect assessments
HCl Clausen
Danish Environmental Protection Agency, COPENHAGEN K, Denmark
The presentation will address different approaches to effect assessments and risk characterisation with the view of a regulator focusing especially on the practicability.Seek simplicity, and distrust it.The simpler the system, the easier it is to handle, and it is generally more transparent. The more complex the testing and modelling is the greater the reality, and also the greater the difficulties in interpreting the results. What should the requirements be for employing the different methodologies and tests.

SS02-8
Addressing the new challenges for risk assessment: An industry perspective
P. Campbell
Syngenta, United Kingdom
This is a comprehensive overview of ecological risk assessment approaches and the high level goals of this paper, such as reduced animal testing and development and promotion of high level ecological risk assessment expertise, should be commended. Key recommendations such as:- adopting higher-tier ecological risk assessment tools; a list of requirements for new innovative approaches before they can be used in regulatory ecological risk assessments; and the need for further research on how to deal with multiple stressors are also well founded. One of more holistic challenges for this document, is the breadth of remit. Currently it encompasses both prospective and retrospective regulation as well as different types of potential chemicals contaminants (eg pesticides and general chemicals), where the issues and the available data sets are going to be very different. Therefore, one way to improve this document would be to consider restructuring the content to provide clear and specific advice to distinctly different situations eg Ecological Risk Assessment for Chemical Registration and Site Specific Ecological Risk Assessment, being 2 potential examples. One of the ecological risk assessment challenges for the pesticide industry is extrapolation of risk assessment conclusions eg between species, different environmental conditions and geographical regions. One of the tools recommended within this document to help with this issue is the expanded use of modelling, and again this is justified. However, another recommendation is for greater uncertainty analysis and application of appropriate uncertainty factor. The industry experience with this option is not so helpful eg Bird & Mammal Guidance Document,

where low toxicity herbicides are still failing the risk assessment. In addition, the current practise of application of safety factors to the results of Mesocosm/pond studies now means there is no regulatory benefit to carrying out such studies. Consequently, a real opportunity is now missed to test, observe and understand potential in-direct and community level aquatic effects, both of which are highlighted as an area where more information is required in this document. The key issue here is there will always be uncertainty within ecological risk assessment and therefore can we really reduce the need for expert judgement as recommended, without having to continue to apply large over precautionary uncertainty factors.

SS02-9

Environmental Risk Assessment - Basic principles, future trends. View from industry employed environmental scientist on the EU (DG Sanco) document 'Addressing the new challenges for risk assessment'

PD Dohmen
BASF SE, LIMBURGERHOF, Germany

The draft EU document represents the state of the art in environmental risk assessment (ERA) including new trends and combining high level environmental protection and realism.The ecosystem services concept offers a suitable approach to determine which impact may be tolerable where and when. Established ERA uses a stepwise approach starting with standardized studies combined with appropriate AF and where needed followed by higher tier assessments and respective adjusted AF. The new EU document follows this approach and presents additional refinements in order to improve the realism of risk assessment. This is certainly desirable; however, the additional data must not become standard requirement.The document describes when to ask for additional information or call for specific risk management actions; i.e. analyse data, include modelling etc. This should be verified by respective field investigations and/or monitoring. Only then specific action should be warranted. This general approach is strongly supported. The document refers to 'indirect effects'. These occur as a consequence of direct effects. If there is low risk of direct effects, then indirect impacts are of low relevance. Only when direct effects are considered acceptable, more consideration should be given to indirect effects.Site specific evaluation will improve realistic ERA. However, this may require a significant amount of additional assessments and should not be misused nationally or regionally counteracting EU harmonized approaches."Trait-based approaches represent a promising tool capable [3DOTS] to complement taxonomically based assessments with functionally based assessment (more significant in ecological terms)". This is valid and relevant; however, this tool is only useful in the regulatory context if it is avoided that individual results from single species will override the trait-based approach. Modelling has been included in ERA allowing to extrapolate risk assessments without the need of excessive (animal) testing; it will also address uncertainties more quantitatively. This should be done in an overall realistic assessment; simply adding up various individual uncertainties has nothing to do with a realistic ERA.

SS03-1

Nanotechnology: Peeking into the Future

S.J. Marshall
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There is great potential for nanotechnology to be applied in a broad range of societal sectors. New applications and product formats are likely to add to current emissions into the environment, e.g. via consumer disposal of domestic products down the drain or via direct emission to, or use in, the environment. Given the developing state of definitions and of characterisation and risk assessment methods, industry is faced with uncertainty over the likely success of new innovation involving nanomaterials and technologies. Rapid progress is needed in these assessment methodologies in order to establish guidance on data requirements and how they should be interpreted for risk-based decisions. In the meantime communication with stakeholders needs particular care to ensure that both research and safety related data can be considered in context of the risks to man and the environment. This presentation will elaborate on developments from an industry perspective.

SS03-2

The regulatory perspective on engineered nanomaterials

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As nanomaterials are used in an increasing range of applications there is growing debate regarding their safe use and possible impact on human health and the environment. There is no specific legislation in the European Union (EU) or theUSAon nanotechnology or nanomaterials, but legislation on worker protection, environmental legislation, chemical legislation, specific products legislation etc. apply in principle to nanomaterials.In the EU, nanomaterials are covered by the chemicals legislation, REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), which addresses chemical substances, in whatever size, shape or physical state, although there are no specific provisions for nanomaterials in REACH. Any introduction of provisions specific to nanomaterials requires the adoption of a definition of nanomaterial. Although in October 2011 the EU Commission adopted the Recommendation on the definition of a nanomaterial, at this stage this does not constitute as yet enforceable legislation.In theUSA, nanomaterials are primarily covered by the industrial chemicals legislation TSCA (Toxic Substances Control Act), pesticides legislation FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act) and for foods and drugs, the FFDCA (Federal Food, Drug, and Cosmetic Act. In the USA, before a new chemical is commercialized a manufacturer or importer must submit a new chemical notification and a determination must be made that it may not present an unreasonable risk to human health or the environment.The presentation will give an overview of the current regulatory perspectives for engineered nanomaterials including a short summary concerning available guidance on safety/risk assessment, and will briefly cover additional international activities.

SS03-3

Advances in methods for analysing nanoparticles in complex environmental media

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The analysis of engineered nanomaterials (ENMs) in the environment is still a challenging task. Advances in the study of the environmental fate, transport, and ecotoxicological effects of ENMs have been hampered by a lack of adequate techniques for the detection and quantification at environmentally relevant concentrations in complex media. The analysis of ENMs differs from traditional chemical analysis because both chemical and physical forms must be considered. Because ENMs are present as colloidal systems, their appearance and physicochemical properties are dependent on the surrounding conditions and may be of transient character. By trying to observe, isolate, and quantify ENMs their physicochemical properties may be changed, making the analysis extremely susceptible to artifacts. The most pressing research needs are the development of techniques for sample storage, extraction/separation, and cleanup that introduce minimal artifacts; to increase the speed, sensitivity, and specificity of analytical techniques, as well as the development of techniques that can differentiate between abundant, naturally occurring particles, and manufactured nanoparticles. The direct and non-invasive detection of ENPs in complex samples is hampered by the presence of sample components interacting with the NPs and/or interfering with the analytical technique. Separation and isolation of the ENPs is therefore necessary in most cases. The basic idea of most currently developed methods is to provide particle sizing capabilities by the use of high spatial resolution or chromatography-like separation techniques. These core capabilities are then combined with material, element- or mass-specific detection. Especially high resolution X-ray, Field-Flow Fractionation and single-particle ICP-MS based techniques have made considerable progress because of their particle size and compositional selectivity. The presentation will cover examples of particle/matrix combinations and discuss the pro and cons of the methodological approaches. It will also address in detail the challenges posed to analytical methods arising from the definition of Nanomaterials issued by the EU commission.

SS03-4

Fate and behaviour of engineered nanoparticles affecting exposure in natural systems

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Research over the past decade has provided a fundamental understanding of the behaviour of engineered nanomaterials in surface waters, sediments and soils. We are well aware that aggregation is an important pathway for most nanoparticles, while for some, e.g. ZnO dissolution has been shown to be an important control on exposure. These transformations will depend both on the physical and chemical properties of the nanoparticle, such as size, surface charge and the presence of coatings, and of the receiving environment, such as ionic strength, pH, and its chemical composition including both dissolved and colloidal organic and inorganic components. Because of the difficulty in working with what could be termed 'environmental concentrations' of nanoparticles, studies of their fate and toxicity have for the most part been undertaken at parts per million concentrations where both the chemical and physicochemical measurements are more reliable, and usually in controlled synthetic water, sediments and soil matrices. Interactions with organic matter have usually been studied using additions of "standard"humic acids.More recently, studies with natural waters, sediments and soils have been undertaken. Here their fate and potential toxicity can dramatically change as a result of aggregation of nanoparticles with natural mineral and organic colloids (heteroaggregation) whose concentrations are likely to exceed those of the nanoparticles, as distinct from homoaggregation with the same nanoparticles. Heteroaggregation will change the surface charge and mobility of nanomaterials and based on the low bioavailability of natural colloids, is likely to reduce toxicity to natural biota. Such reactions will also occur during wastewater treatment (as will chemical transformations, e.g. sulfidation of silver nanoparticles) and will likely contribute to the ultimate transfer of nanoparticles to sediments or to their immobilisation on the soil solid phase. Examples will be presented.The challenge will be to link the exposure concentrations determined under these more realistic environmental scenarios to bioaccumulation and potential toxicity, so that risk assessments and life cycle models can provide more reliable predictions for environmental managers.

SS03-5

Challenges in determining the ecotoxicological responses of engineered nanomaterials

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The ecotoxicology tool box contains a myriad of different bioassays and toxicity tests that use a variety of biological end points. These responses are from different levels of biological organisation from the molecular level to whole organisms; which in turn may be extrapolated to imply population level effects. However, while there is general agreement that many of the existing regulatory test methods may be adapted or modified to work with nanomaterials, less attention has been given to the underlying biological responses. This paper aims to critically evaluate the scientific rationale behind the selection of biological responses as end points for ecotoxicity tests with nanomaterials, and asks whether or not all the important biological responses have been considered in the testing strategy. Ideally, a mechanistic explanation should associate any given biological response with the exposure, but this understanding is lacking for many materials and organisms. Biological responses are time-dependent and for many materials the sequence of exposure, bioavailability, uptake kinetics, and the onset of the biological response has not been verified. An association between the presence of the nanomaterial in the tissue and the biological response (the target organ approach) is not the only paradigm to consider, with secondary toxicity and latent effects also possible. Evidence is emerging that some of the traditional end points in regulatory tests, such as growth or survival, may not be the most sensitive measurements for nanomaterials. However, alternative biochemical, genetic, and other molecular assays that rely on the presence of the test substance being inside the cell may be less sensitive than expected if the nanomaterial is not available to the internal compartments. It is therefore vital to understand the body distribution, target organ and sub-cellular localisation of the materials in order to identify the relevance of each response. Finally, there are many challenges for determining ecotoxicological responses in field-collected samples from real ecosystems; including linking exposure with a nano-specific biological effect(s), and differentiating the nano-effect within a mixture of chemicals.

SS05-1

Human needs, biodiversity and ecosystem services - contradiction or common grounds? Facing sustainability challenges at the third millenium' Introduction to the problem:

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The world population is growing faster than in the last century. In particular, food commerce has been increased to satisfy increasing demands of their populations, resulting in a less controlled grow of food production, particularly food commodities. This higher demand of commodities has been partially satisfied by improving the production technology but also increasing cultivated areas, which result in the use of areas previously occupied by wild biota. Modern agricultural practices make intensive use of agrochemicals to warrantee both productivity and health of cultivars. On the other hand, cattle production uses veterinary drugs, accelerated grow systems (feed lot and similar), sometimes using non authorized anabolic products.Within this framework, South America (SA) is probably the area showing the highest and fastest increase in food production, which result in almost uncontrolled augment of areas dedicated to the production of soya and crops. Particularly Brazil and Argentina but also Uruguay and Chile have been strongly increased their national incomes arising from the exportation of food commodities, in addition to a growing production of bio-fuels. The increase of international prices for soya and maize have been the main argument used for small and big farmers to increase the cultivated area, affecting native forest, spreading agrochemicals in the proximity of cities, controlling pests and weeds but also affecting non target organisms.During this short introduction some figures on the growing demand on food commodities, as stated by FAO (UN) will be presented, with particular emphasis in the production of Latin American Countries (LAC). The main goal of this introductory speech is raising questions on the environmental cost that this increased production is causing in comparison with economical benefits arising from the exportation of food commodities from LAC.

SS05-2

The expansion of agricultural frontiers and its consequences on the environment. The case of Argentina during last decades

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The agricultural expansion and intensification in South America, particularly inArgentina,BrazilandUruguay, has triggered negative changes in the environment. The negative effect of human intervention on habitat and biodiversity would have increased and the water-use efficiency decreased. However erosion and pollution risk are today lower than those of the mid-20th century. Regarding agrochemicals, quantities and qualities of pesticides used inArgentinavary with the productive system. Pedological and climatic characteristics of the Argentinean regions determine the kind and extension of agricultural activities. Extensive soybean and wheat productions are concentrated on the Rolling Pampa region and account for the 80% of the total arable land of the country. This system is based on the direct seeding technique with the application of the herbicide glyphosate, and technical endosulfan and a-cypermethrin as insecticides. On the other hand, Patagonian agriculture is almost exclusively based on the fruit and wine production concentrated mainly on theRio Negrowatershed. The historical and current use of pesticides in the area is reflected on the occurrence of organochlorine pesticides in biotic and abiotic matrices from theRio NegroValleywith a clear predominance of residues of DDTs followed by endosulfans. The intensive and increased agriculture developed inArgentinaduring the last 20 years lead to different effects on biota. Mass mortality of Swainson's hawks occurred inArgentinadue to monocrotophos during 1995-96 and recent investigations in thePampasshowed that bird species richness tends to correlate negatively with annual crops. Non- target species from other ecosystems like the aquatic could suffer the negative consequences of the pesticides. Concentrations of chlorpyrifos and cypermethrin that exceeded the water quality criteria for freshwater environments have been detected in runoff and stream water of small agricultural streams in the main soybean area ofArgentina; associated to 100% mortality of aquatic macroinvertebrates. Environmentally relevant concentrations of endosulfan have been demonstrated that exert oxidative stress, genotoxicity and histological damage in native fish and wetland macrophytes in laboratory experiments. Therefore, residues of this pesticide in freshwater ecosystems are expected to damage wildlife at organism or even more at population level.

SS05-3

THE SCARCE CONSOLIDER PROJECT ON WATER SCARCITY IN IBERIAN RIVER BASINS

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Water resources in Spain are subjected to rising pressures, related to the socioeconomic activities of an increasing human population, expressed by accelerated land use changes, and the specific climate characteristic of Mediterranean countries. The main panels on climate change predict a future scenario of increasing frequency of floods and extended droughts in the Iberian Peninsula, mostly in the Mediterranean basin. This will be added to the currently existing problems, and will probably affect the available water resources, their quality, the functioning of associated ecosystems, especially rivers and their aquifers, and the ecosystem services they provide.
In such context, SCARCE is a project that aims to describe and predict the relevance of global change impacts on water availability, water quality and ecosystem services in Mediterranean river basins of the Iberian Peninsula, as well as their impacts on the human society and economy. Hence, the project has assembled a multidisciplinary team of leading scientists in the fields of hydrology, geomorphology, chemistry, ecology, ecotoxicology, economy, engineering and modelling, in an unknown effort in the CONSOLIDER framework. The project also considers the active involvement of Water Authorities and other relevant agents as stakeholders.
SCARCE has two complementary objectives. The first tackles basic research questions and will define the long-term patterns and the mechanisms that operate in the hydrology, water quality, habitat dynamics, and ecosystem structure and function of Mediterranean basins. The second objective is related to the effects of climate and human footprint (taken both as key elements of global change) that provide on the ecosystem services, rivers and streams, as well as the urgent need to implement and eventually refine the water management policies demanded by the EU Water Framework Directive (WFD). Therefore, the project emphasizes linking basic research and management practices in a single framework. The project has the external support of several Water Authorities and stakeholders. SCARCE is structured across a series of Horizontal and Thematic Work packages that coordinate the various scientific goals, as well as their interactions. These WPs deal with data collection (WP1), hydrology (WP2), sediment transport and river channel morphology (WP3), chemical and biological quality (WP4), ecosystem processes (WP5), modelling (WP6), socioeconomic scenarios (WP7), ecosystem services (WP8), river management (WP9) and coordination (WP10).

SS05-4

Emerging organic contaminants in food: a global sustainability challenge

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There are many thousands of chemical substances in food; most of them being of natural origin. A number, however, are man-made and arise from the use of agrochemicals, or due to pollution of water, soil and air, or occur during food preparation/processing. In addition food may contain biological contaminants. A range of additives may also be added for a variety of purposes (e.g. enhance the flavour, colour, improve stability etc).
Therefore, while consumers expect the food that they eat to be safe, as consequence of industrial development, pollution, and the climate change cause the variety of food contaminants to increase. Currently one of the great challenges in food safety is control the risks associated to mixtures of contaminants, which continuously are changing. Among the most prominent groups of emerging food contaminants can be considered industrial origin contaminants as perfluorinated compounds (PFCs), polybrominated biphenyls (PBBs), the new generation of pesticides, plasticisers, siloxanes, and nanomaterials can be considered. Many of them are of particular concern because can cause severe damages in human health, for example some of them are suspected to be cancer promoters. Other of selected compounds have been related to endocrine disruptor effects, or can be accumulated and biomagnified through the food chain.
In this talk, we will present a summary od current situation, as well as, the results of different research work carried out in our group investigating the presence of emerging contaminants in food. The strategies for their analysis including sample preparation, separation and detection will be presented. The results in typical food baskets of different countries will be compared. The main sources of the selected groups of contaminants will be discussed together with their toxicological data and concentrations reported during the last few years by other authors.

SS05-5
Impact of agrochemicals on the food chain. Research needs to guarantee safe and sustainable food production at the third millennium
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Consequences of the intensive use of agrochemicals have lead to detect presence of these products in edible fish and, sometimes, in fruits and vegetables. Though levels found are still bellow FAO guidelines for safe human consumption, there are many issues needing attention from research community. Particularly, the passage of single agrochemicals or their formulations from plant-soils to higher animals (cattle, etc.) and aquatic systems, impacting fish and other edible products is a topic of concern in South America. Also transport phenomena of these agrochemicals, which are affected by changes in the soil coverage (deforestation), need to be addressed in the near future.So far, Environmental Toxicology in developing countries, dealing with a rapid increase of agricultural frontiers, need to urgently address the above mentioned questions. Regional and international cooperation should be necessary to facilitate studies as well as to spread the concept of sustainable food production for future generations.

SS06-1
Historical and regulatory background of soil quality assessment
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From the 70-ties of the past century onwards concern has been raised about levels of contaminants in the environment. Main sources of contamination are industrial processes, waste disposal and intentionally applied chemicals in agriculture, such as fertilizers, pesticides and veterinary drugs.Regulations dealing with the prevention and remediation of contaminated soils are aimed at the soil as an environmental compartment (soil protection act), industries (environmental regulations), chemicals (REACh, pesticide regulation, Nitrate directive) or waste management (Waste Directive).Policies to prevent and remediate soil contamination use prognostic and diagnostic instruments. Prognostic instruments include soil quality standards and predictions of environmental concentrations of chemicals to manage (intentional) emissions of contaminants. Site specific ecological risk assessment (ERA) is a diagnostic instrument that also uses quality standards, such as intervention values, combined with other lines of evidence. Since the 90-ties species sensitivity distributions (SSD's) have been increasingly used in prognostic and diagnostic instruments, to determine e.g. safe levels and intervention values for contaminants. SSD's seem less suitable for contaminants that have a very specific mode of action, essential elements and nutrients.Some prognostic instruments, for instance for pesticides, may include higher tier testing in case predicted environmental concentrations exceed safe levels.More lines of evidence in site specific ERA may include observations from mesocosms and field tests, as well as ecological knowledge on the role and function of sensitive species in the environment. A Triad approach is often recommended, in which chemical, toxicological and ecological data for a contaminated site are assessed along converting lines of evidence.The European Soil Strategy (2006) has identified 7 additional soil threats, besides contamination. These threats refer also to, biological and physical aspects of soil. The Soil Strategy stresses the importance of soil functions by explicitly listing these functions as protections goals. The 'soil functions' largely comply with the definition of ecosystem services, a concept that gains an increasingly central role in environmental assessments. The potential of this concept for assessing soil quality will be briefly addressed.

SS06-2
Soil ecology and soil quality relationships
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Whenever dealing with environmental hazards, quality assessment of the respective compartment has been heatedly debated. Buzzwords and concepts have repeatedly changed names (e.g. critical loads, critical thresholds, tipping points), yet the key issue - what is a healthy system (here: soil)? - has never been thoroughly conceptualised. Is there an ideal species number, community structure? How do we adequately compare soils with different climates, different keystone species? How do we measure resilience? What is the role of redundancy (which appears to be specially pronounced in soils)? I will summarize theoretical approaches, scrutinize some frequently used endpoints and quality measures such as BBSQ or PICT and discuss their pro's and con's. With respect to available and desirable data I will particularly stress the relevance of various test systems and endpoints, short-term versus long-term monitoring data as well as natural stressors. Trying to structure everything in a logical way and wrapping it with my own considerations, I hope being able to give some new impulses for soil quality assessment.

SS06-3
Predicting or measuring exposure and (bio)availability assessment
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Predicting or measuring exposure and bioavailability of contaminants in soils has progressed significantly in the last 10 years. For metals, robust methods to determine speciation of metals in both the solid phase and in soil porewater are now available. We also know that toxicity of metals is moderated by competitive ion effects at the receptor surface, and these interactions as well as metal partitioning between solid and solution phases can be predicted and/or modelled. "Bottom up"or mechanistic approaches to predict metal toxicity across a wide range of soils have found less success in regulatory implementation than "top down"or empirical methods, largely because of the simplicity of data requirements and ease of calibration and validation of the latter. It is important that the degree of complexity and data requirements of frameworks needed for Tier 1 risk assessments or contaminant screening values be borne in mind during development. Direct measurement of contaminant bioavailability is often regarded as a competitor to predictive models, but it should be realised these are best suited to different levels of the risk assessment process. Great improvements in measures of metal fate, exposure, and bioavailability in soils are now possible with advances in instrumentation and techniques that often now allow rapid and/or in situ measurements that will greatly enhance not only risk assessments but also could assist with risk management processes (remediation).

SS06-4
Natural stressors in ecotoxicological studies and ERA: do they matter?
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Although already some 20 years ago scientists were warning that ecological risk assessment (ERA) based on simple (eco)toxicological essays may be seriously biased, this is still the most commonly used methodology. Although the need for chronic and sub-chronic tests has been recognized to some extent in recent years, what certainly improved the confidence in ERA, these tests are also performed under standard constant laboratory conditions. Moreover, even if these conditions are usually called "optimal", this is not necessarily true. First of all, however, organisms are exposed to a whole range of suboptimal conditions in their natural environment, and these conditions are perpetually fluctuating. Organisms are, thus, exposed to a number of different stressing factors which, irrespectively of any possible pollution, affect individuals and populations. The important question from the ecotoxicological point of view is whether these non-chemical stressors can significantly bias the predictions of ERA. In recent years the number of studies showing significant and environmentally relevant interactions between chemical and non-chemical stressors have increased substantially. For soil environment, temperature, drought, food supply, and pathogens appear to affect toxicity of chemicals. On top of that, also chemical factors of natural origin, such as pH and organic matter contents, can modify the toxicity of pollutants and, most probably, the interactions between toxicants and non-chemical factors themselves, making the picture a whole lot more complicated and difficult to study. Despite these difficulties, it seems that neglecting the interactions cannot be justified any longer. This is proved not only by laboratory studies indicating on significant interactions, which always can be questioned from the point of view of their relevance to ERA, but also by field surveys which actually show substantial discrepancy between field observations in polluted environments and predictions derived from laboratory bioessays. The authors of a recent paper devoted to this problem concluded that " understanding the effects of toxic agents in a complex environmental gradient may require more research on the interaction between toxicity

and environmental factors "(Frouz el al. 2011). I subscribe to this point of view and will review the arguments towards modifying current ERA approaches to include more complicated experimental designs, at least at higher-tier assessment procedures.

SS06-5
Microbial, single species, and higher tier test for predictive assessment of chemical effects on soil quality
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Since 1984, the ecotoxicological hazard potential of chemicals in soil has to be evaluated in Europe andNorth America. During the 1990's and early 2000's, there was considerable research undertaken to develop soil ecotoxicological tests (e.g. SECOFASE project; Environment Canada soil methods program) which used plant species and different soil invertebrates, including earthworms, enchytraeids, nematodes, collembola, staphylinid beetles, mites, centipedes, millipedes and isopods. Semi-field test methods such as the Terrestrial Model Ecosystem (TME) and earthworm field collection have also been developed and standardized through ISO and OECD. Within the past decade, a number of these toxicity tests have been standardized by ISO, OECD and Environment Canada to allow the assessment of sublethal and/or chronic effects on organism growth, behaviour and reproduction. There has also been great methodological progress in tools for assessing the influence of chemicals and contaminant mixtures on ecological soil functions and microbial community biomass, activity and diversity. In Europe, South America andNorth America, these biological test methods have been further standardized for use in the area of soil quality assessment and remedial decision-making at sites where there is complex contamination in natural surface soils. However, adapting biological methods that initially were standardized for testing of individual substances has been a challenge. There is also recognition that endemic soil microbial communities will vary from site to site. To ensure the generation of high quality toxicity test data for effective application in environmental regulations or directives, standardized methodologies, laboratory quality assurance and the proper data interpretation is needed. Overall, the quality of methods, testing laboratories, and data generated ensure that decisions regarding the protection of the environment are made using the most appropriate science. This presentation will provide an overview of existing standardised test methods, focussing on invertebrates, plants and microbial community assessment methods.

SS06-6
Can we realize tangible benefits from using complex 'omics tools when assessing soil quality?
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'Omics approaches are no longer novel but rather serve as powerful tools which can substantially enrich and enhance ecotoxicological investigations. Some proteomic and metabolomic methods have exploited for approaching 20 years whilst after a decade of the exploitation of microarrays based gene measurements we may soon see this technology retired in deference to the more robust New Generation Sequencing based digitial transcriptomics. The maturation of some of these technologies provides an opportunity to appraise what has been learnt so far during their application for chemical hazard assessment. An appraisal of this type considering aquatic ecosystems, and especially fish (Fent and Sumpter 2011, Aquat. Tox. 1055, 25-29), concluded that despite some notable technical achievements and scientific advances, the application of profiling techniques to aquatic ecotoxicology was not yet sufficiently proven for application in either prospective risk assessment or routine environmental monitoring. In this paper, we will discuss the current status and application of systems biology methods for assessing the biological consequences of soil contamination. The analysis will start from an assessment of the lessons learnt from traditional single biomarker analysis conducted at the Avonmouth smelter contaminated site (e.g. expression analysis for an individual gene or protein) both in terms of absolute sensitivity of gene expression measurements and also variation in biomarker responses. An understanding of biomarker sensitivity and variability can lead to hypotheses related to micro-evolutionary processes that can be tested either through exquisitely targeted analysis or genome wide screening. Such analyses can use standard methods or the emerging potential of Next Generation Sequencing. Although often forgotten in the push to move to expression analysis, the genome resources generated as precursors to expression profiling can in themselves offer a resource from which to identify potential mode and mechanisms of action coupled to the biological effects associated with chemical exposure. Linking wider gene expression analysis to physiological observations can provide support for physiological models that categorise chemicals according to their effects on relevant endpoints. Finally, the holy grail of diagnostic based monitoring can become a reality provided that the test case is clearly defined and the molecular assessment is supported by sound environmental chemistry and anchored to physiological analysis of the monitored population(s).

SS06-8
Case study 1: prognostic - risk assessment of plant protection products
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A Dutch working Group drafted a proposal for risk assessment of persistence of plant protection products in soil. The proposal was evaluated for four substances and a metabolite using public available data. A tiered approach was followed for exposure and effect assessment. In this contribution it is discussed whether sufficient knowledge (and methods) is available to ensure the basic principles of a tiered approach for in-soil risk assessment. For the effect assessment protection goals were defined. The relation between the protection goals used and soil health status is discussed.

S06-9
Site-Specific ecological risk assessment. Case-study 2
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The decision supporting and integrating assessment tool, TRIAD, is used site-specific on PAH- and heavy metal contaminated sites in Denmark. The various aspects of the TRIAD approach are used on a set of chemistry-, ecotoxicology- and ecology related data collected among others in the EU project "Development of a decision support system for sustainable management of contaminated land by linking bioavailability, ecological risk and ground water pollution of organic pollutants"or in short "LIBERATION". The presentation includes examples on how to scale and integrate the results from various scientific disciplines.

SS07-1
The decline of the honeybee: a modelling approach
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Insect pollinators play an essential role in crop production with an estimated economic value of about 14.2 billion Euro in the European Union. Among those, honeybees belong to the most important pollinators. In Europe and North America a decline in the number of managed honeybee colonies is reported which may have a severe ecological and economic impact. There are indications that the increased colony losses are not caused by a single factor but by an interaction between several agents. To better understand these interactions we have developed a computer model, simulating the dynamics of a single honeybee colony. In order to keep computational time low but to allow for flexibility in the decision-making, we combined a cohort-based population model with an agent-based foraging module. We included the varroa mite as a bee parasite which acts as vector for two different bee viruses: Deformed Wing Virus (DWV) and Acute Paralysis Virus (APV or ABPV). Crop maps defining the availability of food sources can be used in a separate, spatially explicit landscape model to allow the application of realistic foraging scenarios with specifically defined nectar and pollen flows over time. In this talk we will describe and explain the model's design and present output for the colony dynamics under scarce and abundant forage scenarios, with and without varroa as well as for hypothetical scenarios of pesticide exposure and effects.

SS07-2
Using population models to determine the impact of herbicides on endangered species: an example with lange's metalmark butterflies at the Antioch Dunes National Wildlife Refuge, California
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The Lange's metalmark butterfly (Apodemia mormo langei) is in imminent danger of extinction due to the loss of its habitat caused by invasive exotic plants which are eliminating naked stem buckwheat (Eriogonum nudum var. auriculatum), its food plant. The butterfly was listed as an endangered species on June 1, 1976. Invasive plants significantly impact the few remaining acres of habitat at the Antioch Dunes National Wildlife Refuge. Plant control measures include mowing, hand pulling, fire, and herbicides. In March 2007, the Recovery Branch of the Sacramento Fish and Wildlife Office asked the Environmental Contaminants Division to design experiments to test effects of herbicide treatments on larvae of other subspecies of metalmark butterfly. Due to the lack of literature regarding butterflies and herbicide direct toxicity and the declines in native butterflies it is quite clear that a study evaluating the effects of herbicides on butterflies was necessary. We have evaluated the effects of three commonly used herbicides, triclopyr, sethoxydim, and imazapyr on a surrogate species, Behr's Metalmark and found that exposure of larvae to these products results in a reduction of the number of adults from 24-36%. The effects of these herbicides on Lange's Metalmark population dynamics were determined using stochastic matrix population models (population viability analysis) and will be discussed in this presentation.

SS07-3

The importance of absorption, elimination and feeding pattern: using toxicokinetics modelling to refine the risk assessment of pesticides to wildlife

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Current risk assessment for mammals is based on external exposure measurements, but it has long been acknowledged that effects of toxicants are better correlated with systemically available dose, which depends on many factors, e.g., bioavailability (fraction of dose reaching systemic circulation or site of physiological activity), absorption, distribution, metabolism and excretion (ADME). Toxicokinetic (TK) models are mathematical descriptions of these processes and can be used to refine risk assessments.In toxicological studies of pesticides and biocides, little internal dose data are routinely generated, and the use of toxicokinetic models in risk assessment for pesticides is relatively new. Moreover, for risk assessment of pesticides, the toxicokinetic (and toxicodynamic) should be interpreted in the context of potential exposure in the field. Therefore, it is key issue to include different timescales of exposure and behavioural factors such as feeding pattern in study.Here, we present a case study for an insecticide. A toxicokinetic model was parameterised using data from a rat study with 14C-labelled material. The number of compartments (tissues) in the model and the complexity of the processes of absorprtion and elimination were investigated. Feeding pattern and avoidance of contaminated food were included in the model as they influence internal concentration and hence risk in natural environments.High variability in kinetic parameters between individuals meant it was necessary to test the effects of different combinations of values for absorption ka and elimination ke rate constants on concentration in the blodstream. This was done for different scenarios, e.g. LD50 eaten as a bolus dose and more natural feeding patterns. Maximum internal concentrations (CMaxBody) were compared across scenarios. Our simulations showed that, irrespective of ka and ke combinations, higher CMaxBody were reached when the LD50 was given as a bolus dose than when realistic worse-case scenarios were used. Nevertheless, the difference between CMaxBody reached after a bolus dose or feeding naturally depended on which combination of ka and ke was used; the lower the ka and ke the closer the feeding body burden came to the bolus dose body burden.Ongoing modelling is underway and the impact of avoidance mechanism on the insecticide body burden will be reported. The possible use of body burden model as a refinement option in bird and mammal risk assessments will be discussed.

SS07-4

Mechanistic modeling of the effects of perchlorate on the thyroid gland and risk to post-natal developing mammals

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Mechanistic models can be used to predict the risk to mammal population from exposure to perchlorate. We developed a physiologically-based toxicokinetic (PBTK) model with diffusion mechanisms governing the uptake and distribution of perchlorate. Compartments include blood plasma, liver, kidneys, gut wall, gut contents, and thyroid and pituitary endocrine glands. The thyroid model describes the binding, distribution, and disposal of thyroxine (T4) and triiodiothyronine (T3) including homeostasis mechanisms. These thyroid hormones inhibit the secretion of TSH by the pituitary, which stimulates secretion of the thyroid hormones. Health risk to mammalian species depends upon the concentrations of perchlorate in consumed food and water. Prenatal and postnatal development may be impacted through perchlorate transfer by the placenta and during lactation. Non-lethal assessment endpoints, such as growth retardation and offspring deformities, define health risk. Several individuals are simulated to obtain population level effects. The model is stochastic, with random variables for residues in water and food items.

SS07-5

Why complexity matters: using ALMaSS for risk assessment of wildlife

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The primary focus of environmental risk assessment (ERA) for non-target organisms is on the direct effect of the toxicant. In some cases what are often termed large-scale sources of variation are also considered (e.g. expected crop distribution). However, these describe only part of the variation that occurs in the real world. Landscapes vary in structure, meaning that the field size and proximity to primary NTO habitats will vary. There is climatic variation driving changes in phenology and behaviour, and management changes in the proportion of crops grown, but also changes in how they are cultivated in time and space. All of these factors can affect the risk assessment. There is also another, difficult to observe, property of real systems, and that is the spatio-temporal dynamics associated with populations, climate, management, and ecology and behaviour, and the potential for feedback loops. These interactions can exacerbate or ameliorate impacts either via local feedback mechanisms e.g. multiple stressors, or by virtue of the spatial population dynamics. This uncertainty is normally considered as stochasticity in ERAs and the factors are often incorporated into a single general term, utilizing a safety factor to account for uncertainty. This, however, robs us of both understanding and predictive power, since probability distribution can only be based on statistical expectations of past events, which do not necessarily account for interactions in the future. An alternative approach capable of dealing with these system properties is agent-based modelling (ABM).ABMs are capable of integrating a range of drivers and actors in space and time and can represent detailed farming operations on a large scale, integrating these with realistic models of animal populations, and expressing each animal as an individual agent. As such they are capable of representing very complex dynamics in time and space. Another key model attribute is the ability of agents to respond to the information they gather from their local environment. This automatically integrates many of the dynamics difficult to capture in traditional models, e.g. source-sink dynamics are emergent properties and do not need to be imposed.ALMaSS is one such model system and is used here to exemplify aspects of complexity in population-level risk assessment for terrestrial mammals, birds, and arthropods exposed to pesticides. Results are used to argue for greater realism and population-level ERA.

SS07-6

Incorporating environmental complexity in assessing chemical risks for soil organisms using mechanistic effect models

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Unlike larger terrestrial vertebrates, such as birds and mammals, most soil organisms have limited mobility and may not as easily be able to seek shelter from environmental extremes or completely avoid contaminated habitats. Yet terrestrial soils are both spatially and temporally variable, and this variability may have an important influence on the susceptibility of soil organisms to toxic chemicals. The most effective way to systematically assess the influence of such environmental complexities on risk is through the use of mechanistic effect models. This talk will provide an overview of the types of mechanistic effect models that have been applied to soil organisms with an emphasis on collembolans. We will summarize the types of questions that have been addressed using models, outline the challenges in model development and implementation, and demonstrate the value added by the use of models compared to traditional methods for risk assessment. One area in need of further work is development of robust predictive relationships between soil organism population dynamics and delivery of those ecosystem services of which such species are key drivers.

SS08-1

Combined Effects of Climate Change and Contaminants on Arctic Ecosystems and Humans

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The Arctic Monitoring and Assessment Programme (AMAP) has produced several scientific assessments documenting the levels, trends and effects of specific groups of contaminants such as heavy metals, persistent organic pollutants (POPs), and radionuclides, and the status, trends and effects due to climate change on Arctic ecosystems and humans. The difficulty of attributing the effects observed to a specific problem or cause triggered an increasing need to analyze the potential combined effects of several contaminants in mixture and also together with the current observed Arctic climate change. Based on this, the project 'Combined Effects of Climate Change and Contaminants in the Arctic' was initiated in 2005. The project has focused on the combined effects of climate change, POPs, mercury and radionuclides in selected parts of the Arctic/sub-Arctic marine and terrestrial food chains and humans. The project was carried out by eight Nordic research groups. The first phase of this project has been completed and an overview of the results is presented here. The research priorities covered the following topics:
1) A theoretical analysis of the effects of climate change on the long-distance atmospheric transport and deposition of several selected POPs and mercury in the Arctic using a 3-D atmospheric chemistry-transport model;
2) Quantitative analyses of POPs in snow and air from Svalbard and Greenland to study the concentrations and mobility of these contaminants in relation to environmental conditions such as temperature and solar radiation;
3) A modeling study in which the effect of climate change, in terms of increased temperature and primary production, on the partitioning and bioaccumulation of organic contaminants in biota at various trophic levels in the Arctic marine environment was investigated and quantified;
4) An analysis of changes in the fallout of radiocesium (137Cs) and mobility in the Arctic frozen/melt terrestrial ecosystems and its uptake in terrestrial food webs;
5) A review of abiotic and biological processes relevant to the concentrations and distribution of mercury in the Arctic environment and bioaccumulation in biota;

6) An analysis of the concentrations of selected POPs and mercury in the blood of several indigenous peoples populations in Arctic Russia as a prelude to a further study of their potential effects on people living mainly on local marine and terrestrial food sources.

SS08-2
Southern Ocean and the Antarctic climate system
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The planetary climatic system is sensitive to the conditions of energy exchange between the ocean and the atmosphere in the Southern Ocean. The interbasin exchange accomplished by the Antarctic Circumpolar Current (ACC) is an important link in the global overturning circulation and also admits anomalies formed in one basin be carried around the globe to influence climate in remote locations. As a result, the Southern Ocean strongly influences climate patterns and the cycling of carbon and nutrients. A reliable knowledge of the quantitative characteristics of energy, mass and gas exchange appears to be a basis for the estimation of possible climatic changes in the near decades. Recent observations suggest the Southern Ocean is indeed changing: Data from Argo floats and research vessels confirm that the Southern Ocean, particularly the southern flank of the ACC, has warmed more rapidly than the global ocean as a whole. The average anomalies for 2005-2011, i.e., water temperature deviations from the mean values in the Southern Ocean turned out to be positive in the upper 1500 m layer with a maximum of +0.2 °Cin the layer between 250 and 450 m. The average for the same seven years salinity deviations from mean values indicate weak water freshening in the Southern Ocean with the largest negative deviations of about -0.04 " achieved at 200 m depth.Strong statistically significant abyssal warming trend in the Southern Ocean between the 1990s and 2000s has been also identified. In addition, the dense bottom water formed near Antarctica has freshened in some locations. The freshening is consistent with increased melt from the Antarctic ice shelves and ice sheet. In recent decades the Antarctic Peninsula and some surrounding ocean areas have warmed faster than anywhere else in the Southern Hemisphere.The role of the Southern Ocean in global heat and freshwater balance, including the stability of the overturning circulation, the stability of the Antarctic ice sheet, the stability of the sea ice cover and the future of Southern Ocean carbon uptake, demands the sustained, multi-disciplinary observations system - the Southern Ocean Observing System (SOOS). There is an urgent and compelling need to make progress in the SOOS development to inform decision-makers confronted with the challenges of climate change, sea-level rise, ocean acidification, and the sustainable management of marine resources.

SS08-3
Persistent Organic Pollutants in Antarctica; System Input from Distant and Local Contaminant Sources
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Persistent Organic Pollutants (POPs) are ubiquitous toxic compounds that are incorporated into food chains with high efficiency. Polar Regions have long been established as receiving environments for POPs. In order to manage environmental contamination by POPs in Antarctica, information regarding system input to this remote region is required.
Here we present the first results of atmospheric input of POPs to the Australian Antarctic Territory in over a decade. Further, we present the first audit of an Australian research base as a local emmitter of newly listed POPs and explain biota accumulation of PFCs in terms of species foraging ecology and the dynamics of the Antarctic circumpolar current.
It has recently been shown that climate change is beginning to mobilise global POP reservoirs. Our findings therefore also provide a baseline for temporal monitoring of how input to this remote region stands to be impacted as global secondary sources are perturbed.

SS08-4
Impacts of black carbon on Arctic climate
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Black carbon (BC) particles are products of incomplete combustion. Major emission sources globally are transport and residential heating and cooking as well as wildfires. BC is the most efficient aerosol specie absorbing visible light and it exerts a warming effect in the atmosphere. The significance of BC is higher in the Arctic than elsewhere. Its atmospheric light absorption is enhanced by the high albedo of snow and ice surfaces in snow covered areas, and the deposited BC particles in snow and ice reduce the surface albedo and accelerate the melt rate. BC has a relatively short lifetime in the atmosphere, in the order of days, which implies that emission reductions of BC rich sources would lead to fast responses in its climate impacts. However, since it is emitted along with other particulate and gaseous species that have multiple cooling and warming effects, the net-effect of all relevant species should be taken into account when designing emission mitigation strategies. Recent assessments indicate that a set of measures targeting BC could reduce the projected Arctic climate impacts significantly. The reductions would also bring about co-benefits for public health.

SS08-5
Climate change and the effects on Arctic animals
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The Arctic is one of the regions that the Intergovernmental Panel on Climate Change (IPCC) believes will be most affected by climate change. Some of the expected and already documented alterations are: thinning of the ice sheet, less multi-year ice, increased river discharge into the Arctic Basin, accelerating melting of glaciers, warmer surface temperatures and greater inflow of Atlantic water masses. These changes may potentially alter species distributions, food web structures and carbon cycling, and subsequently tropho-dynamics and transport and uptake of POPs into and within the Arctic.
The Arctic environment is also characterised by high seasonality in light intensity, primary production, food availability, lipid concentration in organisms, migration of organisms, and ice cover. These 'naturally occurring' factors influence the availability of POPs and their uptake in the organisms and in food webs.
In the international polar year project 'Contaminants in Polar Regions (COPOL)' we were able to study the links between climate and contaminants. The COPOL project has shown that accumulation of POPs in marine organisms varies with year and season. However, the seasonal pattern in accumulation and magnification differs depending on the chemical and group of organisms involved.
In all species of zooplankton studied, POP concentrations decrease from May to October. This coincides with decreasing POP concentrations in seawater and increasing lipid stores in the plankton during the same period. Investigation of the accumulation and magnification from zooplankton to fish and birds generally identified July as the month when magnification was greatest, since POP concentrations in the fish species and black-legged kittiwakes were highest in July. The fact that POP concentration in zooplankton was observed to decrease from May to October further underscored the magnitude of increased accumulation through the food web. These results indicate that estimates of the uptake and accumulation of POPs are dependent on the time of sampling (i.e. season).
The level of POPs in marine birds varies between years. In kittiwakes (pelagic feeder) the levels of PCBs and DDE were 50% higher in 2008 compared to 2007. In common eider ducks (benthic feeder) concentration levels were reduced by 50-60% from 2007 to 2008. This shows that diet can exert a great influence on contaminant load. Concentrations of POPs continue to be found at high levels in top-level predators such as glaucous gulls, ivory gull and great skua. Studies investigating effects show that these species are influenced by the current contaminant loads.

Knowledge about the significance of seasonality is essential to be able to differentiate between seasonal variation in accumulation and magnification of POPs versus alterations caused by climate change. Identifying possible alterations caused by climate change in a seasonal environment is a complex and difficult task. Clearly, climate change has the potential to alter POP concentrations in the Arctic. However, the degree to which these concentrations will change, and whether a warmer climate will bring a net increase or decrease of POPs in the Arctic, is still uncertain.

SS09-1
Designing an ecosystem approach for ecological risk assessment of radiation: a path forward for radioecology
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The current approach to ecological risk assessment of radiation based upon "reference organisms"evolved from the radio-toxicological methodology designed to support the development of the system of human radioprotection (concept of "reference man"). Exploiting knowledge on the mechanistic aspects of radiation effects on living matter, it integrates therefore classical ecotoxicological data for some test species along different effect endpoints gathered for individual organisms. As such, it relies exclusively on

the dose-response relationships at the organism level. The approach is limited in light of the extent of both, biodiversity and interaction among species in the environment. Founded essentially on organism-related data, such a method cannot fully meet the actual environment protection objectives that have been set, in the vast majority of situations, at the population- and ecosystem-levels. Furthermore, perturbations induced by stressors within ecosystems cannot be entirely grasped from an exclusive toxicological understanding of the stressor's interaction at the organism level. Such effects only act as triggers of perturbation, which propagate within ecosystems, with ultimate responses that may differ radically from those observed at the organism-level (due to non-linear behaviour of complex inter-population relationships). Also, inter-population relationships, such as predator-prey, are capable of mediating indirect effects by means of which the population actually exposed to the stressor may not be the most affected. This is particularly relevant when considering the long-term ecological effect of chronic exposure to toxicants, like radiation, where damage may not be most due to the direct radiotoxicological effect of radiation per se (upon individual organisms), but rather to the build up of imbalances between interacting populations within ecosystems as a result of differences in their sensitivity to radiation. This communication, therefore, will discuss the wide array of justifications supporting to widen the scope towards an "ecosystem approach"for ecological risk assessment of ionizing radiation, as already adopted in other areas of environment protection (biodiversity, halieutics, etc.).

SS09-2

Are we discriminating against ionising radiation? A comparison of environmental risk assessment for radiation and chemical pollutants

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SS09-3

Are adverse effects of uranium mainly due to its chemotoxicity or its radiotoxicity?

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The ecotoxic profile of uranium (U) has not been studied extensively for non-human biota, particularly for aquatic invertebrates. The toxic action of U in organisms potentially originates from both its chemical and radiological properties, the latter depending on the specific activity of U isotopes and their associated energy radiation. However, information about distinguishing the hazardous effects of its chemotoxicity and radiotoxicity on aquatic organisms is scarce.The main aim of this study was to identify the contribution of the chemotoxicity and the radiotoxicity to U effects on mitochondria and the oxidative balance using transcriptional responses (mt, sod(Mn), cox1, atp6, 12S) and enzymatic activity as endpoints (SOD, CAT, GPx, GST). The opportunity was also taken to evaluate the sensitivity of the used biomarkers by comparing the impacts on the different biological levels of organization after a low contamination level.Therefore, groups of crayfish Procambarus clarkii were exposed for 4 and 10 days to either 30 µg/L of depleted uranium (DU) or 233U which only differ from each other in their specific activity (DU =1.7[GREEKX]104 Bq.g'1, 233U = 3.57[GREEKX]108 Bq.g'1) and hence in their radiotoxicity. The chosen U concentration is close to some environmental values measured near U mining sites and is twice higher than the world health organization recommendation value for drinking-water. U accumulation levels were measured in different organs (gills, hepatopancreas (HP), stomach, intestine, green gland, muscles, and carapace) whereas biological effects of the different types of U were evaluated only in the gills and the HP, because they provided sufficient amount of tissues for conducting various analyses on the same organ. In order to evaluate the radiotoxicity of both DU and 233U, internal dose rates were calculated with EDEN-2.2 software in the HP for the two sets of experiments.Results showed a significant U accumulation in organs of P. clarkii and some effects on the studied biological parameters (mitochondrial damage and antioxidant response). But despite the huge difference of the specific activities (21000x) between DU and 233U, few significant differences in biological responses were noticed for these two pollutants, indicating that the radiotoxicity was low compared to the chemotoxicity in our experimental conditions. Finally, endpoints measured by genes expression levels gave more sensitive responses than those expressed by enzymatic activities.

SS09-4

Importance of the biological role of elements and the trophic level of organisms for transfer of radionuclides to biota

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New requirements in environmental radiation protection imply an increased need for estimations of exposure to biota from radionuclides by risk assessors. A large number of radionuclides that will require assessment in combination with knowledge gaps and complex relations for many radionuclides concerning uptake in biota increases the need to make generalizing assumptions in risk assessments. If an element is biologically essential for an organism this may increase the likelihood of it being taken up, e.g., from food or soil. By that principle, a radioactive isotope of an essential element or a radionuclide with similar characteristics as an essential element might be more likely to be taken up by and accumulated in an organism than a non-essential element. Furthermore, the position an organism has in the food web, i.e., its trophic level, can affect its uptake of radionuclides. For example, uptake pathways for primary producers and consumers vary greatly.This study explores the importance of the biological role of elements and trophic level of organisms for the transfer of radionuclides to biota. The concentration ratios (CRs) for radionuclides and reference organisms in the ERICA tool (July 2007) that originate from empirical data and the new CRs compiled by IAEA and ICRP in the Wildlife Transfer Database (June 2011) for terrestrial, freshwater, marine and brackish ecosystems were used to study patterns in the transfer of radionuclides to biota. The CRs were divided into three element groups on the basis of their biological requirement (major essential, trace, and non-essential). Further, the reference organisms were divided into groups of trophic level (primary producers, consumers etc). The results show that CR values are different for the ecosystems. Moreover, results suggest that for terrestrial biota radioisotopes of essential elements and radionuclides with similar characteristics as an essential element are more likely to be taken up than radioisotopes of non-essential elements. In aquatic ecosystems trace elements have highest CR values. Furthermore, primary producers in all ecosystems and primary consumers in aquatic ecosystems have higher CR values than higher trophic levels. Recognizing the large knowledge gaps for many radionuclides and organisms concerning transfer to biota this type of grouping of elements and organisms can save resources in future risk assessments.

SS09-5

Effects of chronic radiation exposure on plant populations

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One of the major difficulties in the implementation of an ecological risk assessment is a lack of knowledge about the effects from chronic low-level exposures to radioactive contaminants. To understand effects of real-world contaminant exposure properly we must pay attention to what is actually going on in the field. However, for many wildlife groups and endpoints, there are no, or very few, studies that link accumulation, chronic exposure and biological effects in natural settings. The results of long-term field observations in the 30-km Chernobyl NPP zone, in the vicinity of the radioactive wastes storage facility (Leningrad Region), at radium production industry storage cell territory (the Komi Republic), in the Bryansk Region affected by the Chernobyl accident, and in Semipalatinsk Test Site, Kazakhstan that have been carried out on different species of wild and agricultural plants are discussed. Although radionuclides cause primary damage at the molecular level, there are emergent effects at the level of populations, non-predictable from the knowledge of elementary mechanisms of the pollutants' influence. Plant populations growing in areas with relatively low levels of pollution are characterized by the increased level of both cytogenetic alterations and genetic diversity. Accumulation of cellular alterations may afterward influence biological parameters important for populations such as health and reproduction. Presented data provide evidence that in plant populations inhabiting heavily contaminated territories cytogenetic damage were accompanied by decrease in reproductive ability. In less contaminated sites, because of the scarcity of data available, it is impossible to establish exactly the relationship between cytogenetic effects and reproductive ability. Radioactive contamination of the plants environment activates genetic mechanisms, changing a population's resistance to exposure. However, there are ecological situations in which enhanced resistance has not evolved or has not persisted. Consequently, there are good theoretical and practical reasons for more attention being paid to the mechanisms by which populations becomes more radioresistant and to those situations where radio-adaptation appears not to be taking place. Since radio-adaptation plays an important role in response of populations on radiation exposure, this process needs to be incorporated into management programmes.

SS09-6

Analysis of bacterial diversity in a Chernobyl contaminated soil by pyrosequencing

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Chernobyl and recent Fukushima Daiichi nuclear disaster revives the importance of understanding the transfer of radioactive contamination in the environment and its ecological consequences. While some studies have been performed on higher organisms, only a few focused on bacterial communities. It is however well known that bacteria play an essential role in contaminant mobility in soils by lowering or enhancing their transfer to other compartments (e.g. water, plant, animals). Radionuclides (RN) contaminants

might also exert toxic effects on bacteria hence inhibiting their role in the transfer. Thus, the objective of this study was to evaluate the impact of RNs contamination at the bacterial community level by the determination of its phylogenetic diversity. Following the Chernobyl nuclear accident, contaminated soils, vegetation and other radioactive debris were buried in situ in trenches. In this area, the trench n° 22 has been a pilot site for the study of RNs migration in soil for many years. In a previous study, the bacterial diversity was estimated on a set of contaminated and control soil samples on this pilot site using a genetic fingerprint method (DGGE). This analysis revealed the presence of complex communities in the soils but did not give access to the taxonomic diversity. To refine the results, an in depth analysis of the same samples has been conducted by a new high throughput sequencing method, pyrosequencing, leading to 19,000 sequences per sample in average. This molecular technique gave access to unprecedented results and evidenced a huge diversity in the soils with 963 genera and 39 phyla represented. The 4 most predominant phyla, detected in all samples, were Chloroflexi, Proteobacteria, Acidobacteria, and Verrucomicrobia. These data demonstrated definitively that a long term exposure to RNs did not lead to the decrease of bacterial diversity as concluded from the DGGE analysis. However, statistical analysis of the pyrosequencing data evidenced a distinction of bacterial community between contaminated and control samples, suggesting the presence of RN adapted species in the contaminated samples. The pyrosequencing data will guide us for the selection of a model bacteria among a collection of 250 culturable isolates retrieved from these contaminated and non contaminated areas. This model strain will be further used for laboratory experiments to study interactions with representative RNs of the trench (137Cs, U, 90Sr).

SS09-7

Bioaccumulation of Fukushima-derived radionuclides by local marine biota

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The earthquake and tsunami related failure of the nuclear reactors at the Dai-ichi power plant in Fukushima caused radiocesium release amounting to the magnitude of petabequerels. Two thirds of this release were absorbed by the ocean, raising concerns of radiocesium (i.e. 134Cs and 137Cs) bioaccumulation by resident marine organisms and subsequent trophic transfer in the marine food chain. During an international cruise in June 2011, samples of water, zooplankton, and micronektonic fish were collected and later analyzed for gamma-emitting radionuclides. To obtain sufficient biological material for radioanalyses , bulk samples of zooplankton (>0.3mm) and micro nektonic communities (>4mm) were obtained by oblique tows using Bongo and Methot nets. The naturally occurring 40K dominated the total gamma radioactivity in the biota. Generally, radioactivity levels in biota were higher nearer the coast, with concentrations reaching 46 Bq kg-1 dry wt for 134Cs and 56 Bq kg-1 for 137Cs. Crustacean zooplankton displayed higher radioactivity levels than gelatinous zooplankton. We calculated the concentration factors for mixed zooplankton at all stations, which ranged from 9 to 352 with an average of 40, by using Cs concentrations in biota and seawater. Taxonomic analyses revealed that while all zooplankton samples were copepod dominated those that also included copepod consuming chaetognaths had higher concentration factors suggesting Cs biomagnification in the food chain. The ratios of 134Cs:137Cs in both seawater and biota samples were ~1, consistent with reports for their release into the coastal ocean. While no 110mAg was detected in seawater, zooplankton but not fish contained elevated concentrations ranging from 0.5 to 21.5 Bq kg-1 dry wt.

SS09-8

The effect of radionuclide contamination of the Yenisei River on cytogenetic characteristics of aquatic plants

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The YeniseiRiver, one of the world's largest rivers, is contaminated with artificial radionuclides released by one of the Russian facilities producing weapons-grade plutonium (the Mining-and-Chemical Combine, MCC), which has been in operation for many years. Aquatic plants are an important component of water ecosystems, which can accumulate high levels of radionuclides and, thus, can be used in biomonitoring and bioremediation. Internal dose rates to aquatic organisms of the YeniseiRiverwere previously estimated for the area near the MCC. The water moss (Fontinalis antipyretica) accumulated the largest artificial exposure dose among the study aquatic organisms. Preliminary results showed that at the MCC discharge site the occurrence of chromosomal aberrations in cells of Elodea was considerably higher than in the control areas. However, plants growing in other parts of the Yenisei, including those with elevated uranium levels, have not been either analyzed for radionuclides or examined cytogenetically. The purpose of the study was to assess levels of radionuclides and to evaluate the frequency of chromosomal aberrations in samples of submerged plants, collected in different parts of the Yenisei River. The following species were studied: Fontinalis antipyretica, Batrachium kauffmanii, Myriophyllum spicatum, Elodea canadensis, Ceratophyllum demersum and various Potamogeton species. Detailed analysis of radioactive contamination of aquatic plants of the Yenisei River revealed large-scale contamination of aquatic plants as far as 250 km downstream of the MCC. About 30 radionuclides, including uranium and transuranium elements, were detected in the biomass of aquatic plants. The highest concentration factors of the major radionuclides were obtained for Fontinalis antipyretica and Potamogeton lucens. Results of cytogenetic investigations of aquatic plants suggest that at the MCC discharge site and downstream the occurrence of chromosomal aberrations in ana-telophase and metaphase cells of the plants was considerably higher (up to 30%) than in the control areas (6%). Cytogenetic studies of Elodea canadensis samples collected at positions with elevated uranium levels (and decreased levels of artificial radionuclides) showed that the overall frequency of chromosomal aberrations reached 18%. Thus, not only artificial radionuclides but also uranium concentrated in the biomass of aquatic plants can be responsible for cytogenetic aberrations observed in them.

SS09-9

Speciation, bioavailability and toxicity of uranium in different Lemna minor growth media

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Anthropogenic activities have led to a local increase of U concentration to levels that pose potential ecological risks. Uranium toxicity is known to depend on its redox state, speciation and physiological form. The speciation of U strongly varies with parameters such as pH, dissolved organic matter, carbonate and phosphate concentrations and water hardness. The objective of this work was to investigate the bioavailability and toxicity of U to the freshwater macrophyte Lemna minor L. using the standard growth inhibition test [1]. However, as the bioavailable fraction of U strongly depends on the medium composition the total U concentration, speciation and growth of Lemna in different growth media was evaluated. Three different growth media were selected based on their previous use in Lemna growth inhibition tests: (i) the OECD medium [1], (ii) K-medium [2], and (iii) a synthetic freshwater [3]. For each of the media the pH, CO3 and phosphate concentrations were varied. As expected the OECD medium with normal phosphate concentrations (13.4 mg/L) sustained growth (figure1A). Changing the pH (figure 1A) or omitting CO3 from the medium did not affected any of the tested growth parameters. On the other hand lowering the phosphate levels in the OECD medium clearly negatively influenced growth rate. In contrast for the K-medium it was shown that lowereing the phosphate concentration did not adversely influence the growth rate (figure1B). Only when no phosphate was added the growth rate was below 0.250 average and as such also below OECD guidelines. Finally none of the tested Synthetic Freshwater compositions could sufficiently sustain growth of the Lemna plants (average growth rate 0.15/day).Towards the bioavailability of U only in the K-medium with phosphate concentrations of 0.5mg/L or below U in solution could be retreived. A dose-response curve for U was set up (0.05µM up to 500µM). In the K-medium with low phosphate concentrations U concentrations from 50µM or higher induced more than 50% growth inhibition in the plants. In contrast in all other tested media similar concentration did not induce growth inhibiting effects probably due to a changed U speciation. The speciation of U in the different media is currently under investigation.[1] OECD, 2006 Lemna sp. growth inhibition tests. Guideline 221[2] Cedergreen, N, et al., 2007 Env. Tox. Chem.. 26(1):149-156.[3] Charles, AL, SJ Markich, and P Ralph, 2006 Chemosphere. 62(8):1224-1233.

SS09-10

Change of radiocesium concentration in tree leaves before and after abscission

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Due to the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident, several large releases of radionuclides occurred in March, 2011, and in early April, radionuclide releases had dropped compared to the amounts released in March. National Institute of Radiological Sciences (NIRS) located in Chiba, where 220 km away from FDNPP, also had radioactive deposition from FDNPP in March. In newly emerged tree leaves collected at NIRS in late April 2011, radiocesium were found although they had not contaminated directly from the deposition. According to the previous reports and our observation1-3), we concluded that radiocesium was absorbed from tree surface, e.g. leaves (old leaves of evergreen trees) and stems; radiocesium uptake through roots would be negligible.
In autumn, deciduous trees shed leaves, and a part of leaves fall from evergreen trees. It is known that some nutrients in grass leaves withdrawn before abscission4), therefore, we thought it would be possible that leaves return radiocesium to tree stems and trunks before leaf-fall. If so, radiocesium remained in trees for a long time period.
Tree leave samples both living and dead were collected from 13 trees (9 species) in November, 2011 at NIRS. Radiocesium concentrations as well as stable elements, e.g., Mg, Al, Fe, Cu, Zn, Sr, Cs, etc., were measured. Radiocesium and stable cesium concentrations decreased in dead leaves than that in living leaves for deciduous trees, while evergreen tree leaves tended to show no change before and after fall. Potassium in dead decreased for all the trees except a gingko tree. Thus, for most trees, Cs in leaves could be withdrawn to the tree body before fall, and which possibly could be depended on tree types and nutritional condition of the tree. More details will be discussed at the presentation.
This work has been partially supported by the Agency for Natural Resources and Energy, the Ministry of Economy, Trade and Industry (METI), Japan.
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SS09-11

Dose-dependent effects induced by uranium at pH 4.5 in Arabidopsis thaliana

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To evaluate the environmental impact of uranium (U)-contamination, it is important to unravel the mechanisms by which plants respond to U-stress. It was already shown that U-exposure at pH 5.5 can disrupt the cellular redox balance and induce oxidative stress related responses in Arabidopsis thaliana plants (Vanhoudt et al., 2008). However, U-speciation and as such its toxicity strongly depend on environmental parameters such as pH. In a previous experiment, it was shown that U-toxicity at pH 4.5 was higher than at pH 7.5. Therefore, we want to investigate dose-dependent effects at low pH.Arabidopsis thaliana plants were exposed to U-concentrations ranging from 0 to 100 µM at pH 4.5 during 3 days. U-concentration, fresh weight, lipid peroxidation and photosynthetic efficiency were analysed. On protein level, the enzymes of the antioxidative defence system were analysed to evaluate the importance of the cellular redox balance in Arabidopsis thaliana plants exposed to U.Results indicate that the U-content in roots and shoots increased with increasing U-concentration added to the nutrient solution. However, there was a low root-to-shoot transfer. Fresh weight of roots and leaves decreased after exposure to 50, 75 and 100 µM U. In contrast, plants exposed to 6.25 and 12.5 µM U had an increased fresh weight as compared to the control plants, which alludes to a hormesis effect as was observed before (Vanhoudt et al., 2008, Straczek et al., 2009).One of the most important physiological processes in plants is photosynthesis. Apparently, the photosynthetic efficiency of plants exposed to 25-100 µM U was increased. This indicates that the photosynthetic system is not damaged but in contrast plants try to optimize their photosynthesis under U-stress.In addition to the reduced biomass production, plants exposed to 25-100 µM U showed an increased lipid peroxidation. This increase indicates an affected membrane integrity and functionality. Assessing antioxidative enzyme capacity of the plants indicated an increased defence against ROS in the U-exposed plants as evidenced by increased activities of ROS scavenging enzymes. The increased activity of guaiacol peroxidase could indicate an increased cell wall lignification as a defence reaction that limits the entry of toxic metals.In conclusion, this study indicates that elevated U-concentrations at low pH can cause important morphological, physiological and biochemical effects in Arabidopsis thaliana seedlings.

SS09-12

Environmental sensitivity as a tool for the risk assessment of the use of nuclear energy

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Approaches to the management of the risk in radioecology have to take into account geographic, climatic, living and dietary habit differences and in more detail ecosystem differences. The understanding of the factors of sensitivity of different environments, populations or geographic areas is important for scientists and policy makers, to set priorities for the allocation of limited resources. Furthermore the identification of vulnerable environments will be valuable in planning the locations of new nuclear facilities.A Task Group on Radioecological sensitivity was organized by the International Union of Radioecology (IUR) in 2007 on the basis of studies of the Radioecological Sensitivity Forum, 1998-2001. The objective was to discuss a standardization to represent the radiological state of the environment following accidental pollution and a scale of radioecological sensitivity of areas, useful in emergency planning and preparedness. The work of the Group continued under the International Atomic Energy Agency (IAEA) EMRAS II Programme, from 2009 to 2011, as Working Group 8 on Environmental Sensitivity. The WG8 focused its studies on sensitive non-urban environments. The aim was to investigate which environments, which components of each environment, and which seasons of the year would be most sensitive to a major release of radionuclides. The overall aim is to aid in the planning and implementation of emergency as well as long-term countermeasures following a nuclear accident.Sensitivity analysis was performed for different environments: temperate agricultural and alpine (Europe andCanada), coastal marine (Nordic seas, North-East Aegean Sea, Thermaikos Gulf Mediterranean Sea), temperate forest (Northern Saskatchewan and Canadian), freshwater aquatic (Norway,Italy, Northern Saskatchewan) and Arctic (Northern Canada).Each environment was to receive the same deposition of 137Cs, 131I and 90Sr. The concentrations of these radionuclides in key environment compartments as functions of time after the event were calculated, and also the doses to human populations who receive most or all of their food intake from the respective environments. Models participating to the work were CHERPAC, ECOSYS, FDMT-RODOS, HealthCanada model, IMPACT, MOIRA-PLUS, NRPA box model, NTUA 3D model.Results are presented, showing as the interaction of environmental but also of social and economic factors plays a role in defining the radiological sensitivity of geographic areas.

SS09-13

Agricultural land management options following large-scale environmental contamination

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The accident at the Fukushima Dai-ichi Nuclear Power Plant has raised questions about the accumulation of radionuclides in soils and the transfer in the food chain. Following a large-scale nuclear accident, the application of countermeasures is a key issue. Numerous countermeasures were developed since the Chernobyl accident and applied on large scale. This presentation discusses countermeasure strategies and their effectiveness and feasibility against the background of the Fukushima Dai-ichi nuclear accident and the agricultural areas affected.Land management options should be selected based on a criteria such as effectiveness; constraints on implementation; wastes generated and waste management options; doses received during implementation; side-effects; cost/benefit considerations; acceptance (stakeholder opinion).Mechanical (soil removal, ploughing) and agrochemical (e.g. application of fertilizers or caesium sorbents) management options will be discussed in terms of their potential effectiveness in reducing the soil-plant transfer but also potential side effects will be highlighted. Specific attention will be attributed to andosols, a prevailing soil group in Japan. In addition phytomanagement options such as food crop selection and cultivation of technical crops (e.g. bioenergy crops, fibre crops) will be briefly discussed. Phytoextraction will be critically examined. Land management options will be evaluated based on available information on soil contamination levels, soil characteristics, food and non-food crop production and conversion systems in Japan and following interaction with institutes belonging to NARO (Japanese National Agricultural and food Research Organisation), and considering the evaluation by international teams of the remediation activities in Japan.For optimizing agricultural management options, a good knowledge on agricultural practice and soil characteristics is required. Alternative land uses for areas were contamination levels are considered too high, need careful evaluation. Planning for agricultural management options requires a holistic approach, considering radioecological, radiological, environmental, economic and socio-cultural and political aspects.

SS09P-1

The concept of radiocapacity: theory and application for estimations of ecological risks in ecosystems

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After the accident in Chernobyl, the most part of Ukraine has been contaminated with cesium-137. Meanwhile, cesium is the closest analogue of potassium that is known as the macro element of great importance. Therefore, 137Cs has turned out to become the inescapable tracer in biological objects of all ecosystems in Ukraine, almost without exceptions. Thus, there is every reason to believe that the monitoring of this radionuclide can be employed for quantitative estimations of robustness of biota in the ecosystems. We suggest the concept of radio-capacity, as the novel approach, for estimation and prognosis of the states of ecosystems biota exposed to unfavorable physical and chemical factors. The radio-capacity is defined as the threshold (limit) amount of 137Cs, i.e. - the amount at which there are still no visible violations in the basic functions of biota, such as the capability to retain the biota biomass and the environmental conditions. Besides, basing on this concept and the theory of reliability, we have developed the mathematical model of the radio-capacity. Comparison analysis of the data for different kinds of ecosystems, including terrestrial, aqueous, forest, grasslands and urban landscapes, demonstrates that the parameters of our reliability-theory model are adequately sensitive to the impacts of ?-irradiation and heavy metals on the biota. We show that both, distribution and redistribution, of the cesium-tracer in aqueous and terrestrial ecosystems neatly follow all essential external factors, like the changes in climate, floods, contra-measures, and so on, as well as they respond to different kinds of contaminations, like the ionizing radiation, heat fluxes from atomic power stations and chemical pollutants. The higher is the radio-capacity factor, the greater is the biota robustness and the less is the ecological risk for the biota. Moreover, we have demonstrated that the ecological risk for the biota inevitably and essentially increases when the radio-capacity of the biota decreases under the influence of the pollutants. Furthermore, we have demonstrated the key role of the reparation and restore processes on exposure of the biota simultaneously to radiation and chemical pollutants. Therewith, the interference of the different factors may change from synergism to antagonism. Thus, it may be concluded that the radiocapacity holds much promise as the unified equidosimetric parameter for quantitative estimations of impacts of different factors on the ecosystems biota. In addition, basing on the radio-capacity concept, we suggest the method of ecological standardization that is prospect to determine the threshold levels of pollutants acceptable for the ecosystems biota, as well as to assess the levels of ecological risks.

SS09P-2

Radiocesium distribution in a bamboo forest

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Due to the accident of Fukushima Daiichi Nuclear Power Plants (FDNPP) occurred on 11 March, 2011, some areas were contaminated with radionuclides. Since one year has been almost passed, we can find largely released, long-lived radionuclides in the terrestrial environment, that is, Cs-134 and Cs-137. The behavior in forests is of interest because large areas are covered by forest in Japan. In this study, we focused on bamboo forest, which is commonly found in Asian countries. Since Japanese use young bamboo shoot as a food materials and bamboo stems as various craftwork products, radiocesium behaviors in bamboo forests are of interest.
We collected stems, leaves, shoots, litter, and soil samples of a bamboo forest (Ibaraki Prefecture) on 8 May, 2012 to see the distribution about 2 months after the large radionuclide releases from FDNPP. The results showed that most radiocesium was on bamboo body and litter layers; the concentrations of Cs-137 in twigs, leaves and litter samples were 0.9, 6.4 and 9.6 kBq/kg-wet, respectively. However, the concentration in soil was 0.5 kg/kg-dry, possibly because of the interception effect by the litter layer. The young bamboo shoots contained some radiocesium due to translocation from old bamboo trees. About 30% of radiocesium was removed from the young bamboo shoot by boiling.
In October, we collected samples from the same forest again. The Cs-137 concentrations in the leaves decreased by ca. 100-fold, and that in the litter layer was ca. 6-fold. Bamboo trees in Japan shed their leaves in late spring, and thus Cs concentration decreased dramatically in leaves. On the other hand, Cs-137 concentration in the soil samples increased by three times; Cs leached from the dead leaves to the soil. Since radiocesium concentration in the bamboo tree decreased, we expect that radiocesium concentration in young shoot will be much smaller in this year than that we observed in the last year.
This work has been partially supported by the Agency for Natural Resources and Energy, the Ministry of Economy, Trade and Industry (METI), Japan.

SS09P-3
The activity of uranium, thorium and some decay products in chickens and eggs
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The ingestion of radionuclides during food consumption can represent major source of radiation exposure to humans. Uranium, thorium and their decay products, are naturally present in the environment and can be transferred to animals and animal products. Even if the background levels in animal products don't represent a significant risk due to the low activities of radionuclides, their evaluation is important. Indeed, the assessment of the magnitude of a contamination due to accidental or chronicle releases of radionuclides depend on the knowledge of the natural activities. The present study aims in the first instance to quantify the activities of uranium, thorium and their decay products in chicken meat and eggs in five regions of the French territory. The highest activities (mBq.kg-1 fresh weight) are measured for radium isotopes in eggs and range between 136 and 190 for 226Ra. In comparison, uranium activities in eggs are much lower and range between 0.51 and 1.30 for 238U. In chicken meat, 238U activity concentrations show higher values than in eggs and range between 1.7 and 9.7. Concerning 232Th, its activities are lower than those of 238U and range between 0.5 and 4.9. Secondly the contribution of the potential sources of the radionuclide for chicken meat and eggs (grain mixture, soil particles and drinking water) are studied in order to calculate the concentration ratios and the transfer factors. These values could enrich the data given by the technical report series N472 of IAEA (2010). In this report the mean transfer values for uranium in chicken meat and eggs (0.75 d.kg-1 and 1.1 d.kg-1 respectively) are only based on two results from the study of Prister (1967). Moreover no concentration ratios are given up to now in the literature for uranium, radium and thorium in eggs and chicken meat. It is important to complete this database, to provide reference transfer coefficient values which could be used to predict the behaviours of radionuclides in the environment.

SS09P-4
Annual intakes of ^{226}Ra, ^{228}Ra and ^{40}K in staple foodstuffs from a high background radiation area in the southwest region of Cameroon
Ele Abiama P., Ben-Bolie G.H., Amechmachi N., Najib F., El Khoukhi T., Owono Ateba P.
Annual intakes of 226Ra, 228Ra and 40K in staple foodstuffs from a high background radiation area in the southwest region of Cameroon
Concentrations of naturally occurring radionuclides 226Ra, 228Ra and 40K were determined in five most consumed vegetables in a high-level background radiation area (HLBRA) in the southwest region of Cameroon. A total of 25 foodstuff samples collected from Akongo, Ngombas, Awanda, Bikoué and Lolodorf rural districts were analyzed by gamma spectrometry. The average activity concentration values of 226Ra, 228Ra and 40K were respectively 2.30, 1.50 and 140.40 Bq kg-1 fresh-weights. The effective dose for individual consumption of the investigated foodstuff types was calculated on an estimated annual intake of such diets for Adults, Children and Infants in the study area. The estimated total daily effective doses from the ingestion of the investigated foodstuffs for each studied long-life natural radionuclide were respectively 0.41µSv for 226Ra, 0.84µSv for 228Ra and 0.71µSv for 40K. The total annual effective dose was estimated at 0.70 mSv y-1. 228Ra (44%) and 40K (36%) were found to be the main sources for internal irradiation which is very likely due to the specific uptake of these radionuclides by the studied plants.

SS09P-5
Incorporating plant physiological pathways to mechanistic modelling soil-to-plant transfer of radionuclides
T. Sauras, J. Casadesus, R. Vallejo
BIORUR is a mechanistic model developed to assess the soil-to-plant transfer of radionuclides. BIORUR algorithms assume that the movement of radionuclides in an ecosystem and inside the plants is through the same network of pathways than nutrient cycling. The fluxes of radionuclides between compartments inside an ecosystem or a plant are estimated from the flux of a nutrient analogue between these compartments, the ratio of concentration radionuclide to nutrient and a selectivity coefficient which compares the affinity of the pathway for radionuclide to that for its nutrient analogue. One advantage of BIORUR algorithms is the flexibility to adapt the model to a wide range of scenarios where the only requirements are that nutrient cycling have been quantified and that each radionuclide must be associated to a nutrient analogue. However the scope of radionuclides is restricted to those which are associated to a nutrient analogue, such as Cs-K, Sr-Ca. BIORUR model incorporates some of the biological processes relevant to radionuclide availability such as plant physiology, mycorrhizal transfer, organic matter mineralisation and micro-organism. In order to study the plant physiological mechanisms involved, we have developed a hydroponics plant growing system in which we manipulate the plant-environmental processes affecting the uptake of radionuclides (i.e. transpiration, plant growth rate, nutrient demand, nutrient supply). The experimental system permits to verify the mechanisms of Cs and Sr uptake already included in BIORUR.

SS10-1
REACH: what can be learned from the first registration deadline?
W. De Wolf
European Chemicals Agency - ECHA, Helsinki, Finland
The first REACH registration deadline for substances produced at 1000 tonnes or more per year, carcinogenic/mutagenic/reprotoxic substances (CMRs) and substances produced at 100 tonnes or more which are classified as R50/53 has passed 30 November 2010. ECHA has started testing proposal evaluations and compliance checks on registration dossiers. In 2011 for instance, ECHA has started 472 testing proposal evaluations and completed 146 compliance checks. This presentation will give feedback from ECHA on the first REACH registration deadline, with an emphasis on environmental hazard and exposure data. Main topics are: 1) amount of data submitted, 2) type of data submitted, 3) quality of the data, 4) feedback from evaluation, 5) lessons learnt and recommendations for the next registration deadline of 31 May 2013, 6) opportunities for scientific developments from data mining from public registration data. Substance evaluation and authorisation will be briefly discussed as well..

SS10-2
REACH and Science
D. Merckel
Environment Agency, Oxfordshire, United Kingdom
This talk will explore scientific work the Chemicals Assessment Unit (CAU) of the Environment Agency has undertaken that is directly relevant for chemical hazard and risk assessment under REACH. Aspects of our work as they relate to the existing legislation, and to research and development in the field of bioaccumulation will be covered.
In the last two years there have been a number of significant revisions for the regulation and testing of PBT (persistent, bioaccumulative and toxic) and vPvB (very persistent, very bioaccumulative) chemicals in the EU. Annex XIII of REACH, which lists the PBT/vPvB criteria, has been revised introducing additional ways to capture substances as being 'PBT-like', or of equivalent concern to PBT substances, even though they do not meet the numerical criteria of degradation half-life, aquatic bioconcentration factor and long-term aquatic toxicity. Some of these additions to the annex will be discussed and illustrated with practical examples that CAU have been working on.
The OECD Test Guideline (TG) for measuring aquatic bioconcentration (TG 305), a very important guideline for both risk assessment and PBT assessment, has been the subject of a revision over the last four years. CAU, on behalf of the UK and in partnership with the Netherlands and Germany, led on this work. The revisions mean that fewer animals can be used in the existing aqueous test in certain cases and, for highly hydrophobic substances, a dietary exposure test has been added. The revised test guideline has more detail for interpreting test results, as our understanding of important factors in laboratory aquatic bioaccumulation testing has grown. The major changes concerning data treatment and interpretation relevant for both exposure methods (e.g. fish growth, kinetics, metabolism, etc) and specific for each method (e.g. kinetic BCF derivation, BCF estimation from dietary study data) will be presented with reference to chapter R.7.10.1 of the REACH technical guidance and desk-based research and development work published by CAU.
Finally, the relevance of the revised test guideline for B testing under REACH and the apparent changing emphasis of B assessment will be briefly explored.

SS10-3
CSR Preparation Experiences - Science Behind the CSR
L.I. Sweet

Lubrizol, United States of America
Important lessons can be learned from the CSR preparation work already conducted for the first REACH registration deadline. While the Chemical Safety Assessment (CSA) and Chemical Safety Report (CSR) documents the conditions under which the risks associated with the use of a substance during its life cycle are managed to a level that is considered safe to humans and the environment, it can also be a reflection of the state of the regulatory science, tools, and resources used to understand chemical hazard, exposure, and risk. This presentation highlights a number of key areas in the CSR where use of the state of the science, weight of evidence, and read across is especially important; including PBT assessments, PNEC and DNEL calculations, environmental modeling, release factors, and others; and provides insights to help industry put the best science forward in complying with this complex legislation.

SS10-4
Updates of dossiers in light of new scientific data: expectations on what is regarded as new in a practical way. What are the problems and challenges for the future?
P. Douben
REACHWise, Nederland
REACH places a duty on registrants to keep their dossiers up to date. Amongst those possible update reasons is new scientific data. This begs the question what the boundaries of this concept are, and what it means in a practical way. Does the discovery in a lab trigger updating obligations or not? In other words what are possible criteria, time line, etc. The issue cannot be seen in isolation but must be placed in the entire framework of operations in which companies operate to meet their legal obligations.

SS10-5
What can be learned from the first registration deadline? Expectations for the next registration deadline
N Nimpuno
ChemSec, GÖTEBORG, Sweden
NGOs had very high expectations on the first registration deadline. At last, large amounts of information on individual substances would be published by ECHA, giving the general public, authorities, scientists, as well as NGOs, access to high quality environmental information for all high tonnage substances. However, the reality has instead caused concern due to delays in dissemination and because important information has not been published, in particular each registrant's name and tonnage, thus making it impossible for non-authorities to connect the submitted information to a responsible company. Further, many registration dossiers have proven to be of poor quality, and there are data gaps for key parameters from higher-tier studies such as endocrine disrupting properties, persistence and environmental fate. The lack of correct and sufficient data in the registration dossiers can adversely affect enforcement, with enforcing activities already strained. For the coming registration deadline in 2013, an emphasis must therefore be placed on correctly compiled registration dossiers from companies and a rapid dissemination of the submitted information by ECHA. NGOs call for the dissemination portal to flag the status of assessing confidentiality claims on data that has been claimed as confidential by the registrants. NGOs also call for other information such as the presence and amount of SVHCs in imported articles should also be disseminated, ideally by function/ product group and volumes. NGOs want to have such information to have input into the processes for dealing with substances of concern, i.e. restrictions, candidate list prioritisation and of uses under the authorisation procedure.

SS10-6
ITS Schemes for Human and Environmental Toxicology in REACH
G. Schüürmann
UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany
Through the implementation of the European chemical legislation REACH, alternative methods have gained an increased importance as non-animal tools for the toxicological assessment of chemical substances. In this context, the 3R principle of reducing, refining and replacing animal testing has resulted in a paradigm shift: While the original idea was to replace a given animal test by a single in vitro alternative (1:1 replacement), the integrated testing strategy (ITS) approach aims at exploiting the combined information generated from several (n) non-animal methods in order to - partly or fully - replace the respective animal experiment (1:n replacement). In the presentation, the ITS concept and its major components covering non-test (in silico) and test (in chemico, in vitro, omics) methods as well as information theory are outlined. For illustrating their way of application, both human and environmental toxicology endpoints are taken, building on respective results as developed within the EU-funded OSIRIS project.1 Particular emphasis is put on the mode of action as guiding principle for extrapolating, and on consensus evaluation as a practical tool to characterize and improve the level of confidence in ITS scheme applications.
[1] OSIRIS. Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Test Information. EU Project, contract no. GOCE-CT-2007-037017, 2007-2011. OSIRIS project website: http://www.osiris-reach.eu/. OSIRIS webtool website: http://osiris.simpple.com/OSIRIS-ITS/welcome.do.

SS10-7
From stress genes to toxic effects and vice versa. A risk assessment perspective
B. Piña
IDAEA-CSIC, Barcelona, Spain
Toxicant-induced changes in gene expression often constitute the very first level of a cascade of events that result in toxic effects at the whole-animal level. Their analysis provides valuable information about the mode of action of the toxicant and, in many cases, sets molecular concentration thresholds below which no effect is predicted. However, and since the analyses of single regulatory events are often perceived as not representative for systemic effects, regulatory toxicology is still reluctant to fully incorporate these technologies. Recent major advances may provide a bridge between molecular responses and whole-organism effects. The ability to characterize effects at the whole genome level allows the identification of targets for each toxicant or for different physiological modes of action. In addition, our ever-increasing knowledge of cell regulatory networks facilitates the systemic interpretation of these coordinated transcriptome changes. Finally, the development and validation of relevant non-animal models for vertebrates and humans, like cell lines, fish and amphibian embryos, or stem cells, diminishes or even eliminates the need for animal testing and allows high throughput screening schemes. In this regard, the combination of gene expression-based methodologies with chemical structure-oriented analyses (as QSAR) may constitute a solid and reliable strategy for the assessment of health risks associated to the thousands of new chemical products released into the market every year.

SS10-8
Chemical Risk Assessment under Chemical Substances Control Law in Japan and comparison with REACH
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The purpose of Chemical Substances Control Law (CSCL) is to evaluate, before manufacture or import, whether or not new chemical substances have properties such as persistence, and to implement necessary regulations, in order to prevent environmental pollution caused by chemical substances that pose a risk of impairing human health or interfering with the inhabitation and/or growth of flora and fauna.

To minimize the significant adverse effect of chemical substances on human health and environment by 2020 (Agreement in the Environmental Summit in 2002), CSCL has been amended in 2009 and implemented in 2011. Under amended CSCL, risk assessment will be conducted in step-wise manner for all chemicals including existing chemicals. In this presentation, chemical risk assessment approach under amended CSCL and the results of comparison between CSCL and REACH will be introduced.

SS11-1
Non-standard methods for environmental endpoints under REACH: scientific challenges & opportunities
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The overall purpose of both the REACH (EC No 1907/2006) and the CLP (EC No 1272/2008) Regulations is to ensure a high level of protection of human health and the environment. Industry has to ensure that chemical substances are used safely.A key motivation for developing REACH was to fill information gaps for the large number of substances already in use in the EU, as for many there was inadequate information on their hazards and risks they pose. REACH registrants have to provide information on the intrinsic properties and hazards of the substance in the registration dossier. The standard information required depends on the tonnage manufactured or imported; the higher the tonnage, the more information needed. In addition for substances at 10 tonnes per annum (tpa) or above, the registration dossier must include a Chemical Safety Report (CSR).New studies using vertebrate animals for REACH registration should only be conducted as a last resort. For information specified in Annexes IX and X (more than 100 and 1000 tpa, respectively), a testing proposal should be approved first before testing on vertebrate animals is done. In addition there are data sharing obligations for registrants of the same substance to avoid duplicate testing using experimental animals. Registrants must first collect and assess all existing data. Then they have to identify data gaps and consider whether they can be filled by using non-standard data before any new tests are conducted. This means that all available information is collected: in vivo and in vitro studies, information from human exposure, information from structurally-related substances (i.e. 'read-across' and 'chemical categories') and predictions from valid (Q)SARs. The non-standard information has to be equivalent to the information obtained from the standard test data. The key point is that the non-standard data must be suitable for an adequate risk assessment to ensure the substance can be used safely and also for adequate classification for hazard communication.This presentation summarises the current experience, discusses the scientific challenges and opportunities, and presents some recommendations for improving the use of alternative methods in the environmental hazard assessment under REACH/CLP.Disclaimer: The authors are staff members of the European Chemicals Agency. The views expressed are solely the authors' views and do not represent an official position of the Agency.

SS11-2

Opportunities for Animal Alternatives Implementation in the Evolving OECD Fish Testing Framework

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The OECD Fish Testing Framework (FTF) was proposed by the USEPA to OECD in 2009. The goals were to review existing fish testing guidelines in the context of regulatory needs and data requirements, address animal welfare concerns and to define a possible testing strategy for fish tests. A workshop in 2010 and subsequent follow-up meeting in 2011 developed a document that focused on assessments of 13 existing/proposed fish Test Guidelines and subjects relevant to fish testing. Chapters were reviewed in detail and future recommendations for action were proposed to address various needs. Flow diagrams exemplify how tests could be integrated into an overall strategy for assessing toxicity of chemicals to fish.

This presentation reviews opportunities for animal alternative approaches in the FTF. These will be placed in the context of in vivo tests required in many regulatory situations for the registration of industrial chemicals, pharmaceuticals, and agrochemicals. The Framework integrates current thinking regarding screening assessments, acute toxicity, chronic toxicity, bioaccumulation, and endocrine disruption determinations. Ample room exists for improved use of in vitro, embryo tests, threshold approaches, and implementation of robust experimental designs. The overall goal is to minimize animal use while maximizing the collection of information useful in hazard and risk assessment, and classification and labelling. Method development needs and future opportunities will be identified throughout.

SS11-3

The validation journey - What is validation and how does it work?

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It is common understanding and a regulatory need that alternative methods to animal tests are reliable and relevant, i.e. validated. According to OECD Guidance Document 34 on the validation and international acceptance of new or updated test methods for hazard assessment, the process of validation establishes the reliability and relevance of a method for the given purpose. Reliability refers to the reproducibility of the method within and between laboratories over time, relevance to its scientific value and practical usefulness, and purpose to its intended application.The presentation will outline the various steps in the validation process: a) the definition of a test method and how to assess its readiness to enter validation; b) the assessment of its transferability, within- and between-laboratory reproducibility; c) assessment of its relevance; and d) the peer review by independent experts (e.g. the ECVAM Scientific Advisory Committee, ESAC). Crucial aspects in the design of a validation study (e.g. number of laboratories, selection of chemicals, number of chemicals to be tested, etc) will be discussed and examples will be given.The role of the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) is to promote the validation and use of alternative approaches and their international regulatory acceptance. It has been formally established by Council Directive 2010/63/EU on the protection of animals used for scientific purposes. In this context, two key bodies have been created over the past year: the PARERE (Preliminary Assessment of Regulatory relevance) network to advise EURL ECVAM on the regulatory relevance of alternative test methods submitted for validation and ESTAF (ECVAM stakeholder forum) to maintain the dialogue with the stakeholders. In addition, the EURL ECVAM is setting up NETVAL, a network of laboratories, which have been nominated by European Union Member States and will participate in EURL ECVAM coordinated validation studies.

SS11-4

Can cell-based systems help predict adverse outcomes in fish?

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Fish cell-based systems hold great potential for deciphering the molecular mode of action of chemicals and, provided the right choice of in vitro model and exposure conditions, may supplement or even substitute fish toxicity tests. Conceptually, if the cell-based responses reflect the initial stage for an adverse outcome seen at the organism level, it must be possible to develop an array of cellular systems suitable to identify mechanisms of action and adverse outcomes. In support of this concept, we have recently demonstrated that a gill cell line from rainbow trout (Onchorynchus mykiss), RTgill-W1, responds with a loss of cell viability at comparable effect concentrations as fish respond with death in acute exposure scenarios for a wide range of organic industrial chemicals with different modes of actions. The gill epithelia is the primary uptake site of water-born contaminants into fish; thus, damage to gill epithelia can be seen as the link to an impairment of the whole organism and eventually death. Transferring this concept to adverse outcomes other than acute lethality certainly requires more sophistication. For example, cell-based models could be used to elucidate the role of epithelial barriers (such as gills, intestine) or tissues (such as liver) in transforming and excreting chemicals. Different cell cultures maybe put together, e.g. on microfluidic devices, to better resemble the interaction of different cell types and transport of biotransformation products and signaling molecules from one compartment to another. Moreover, molecular mechanisms of toxicant action can be identified as part of a tiered testing approach but as well, cellular parameters, such as ATP production or proliferation should be explored as potential indicators for, e.g. reduced growth in fish. If these systems are combined in an intelligent way and systematically linked to chemical concentrations and response pathways and outcomes in fish via computational approaches, they provide a foundation for the development of a "virtual fish".

SS11-5

In-vitro methods for bioaccumulation: a modular approach including passive uptake and metabolism assessment

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Predictive tools for the assessment of bioaccumulation are critical components in an overall strategy for assessing bioaccumulation potential. This presentation reviews the potential of in-vitro test systems for improved prioritization and assessment of potentially bioaccumulative chemicals. The toxicokinetic processes of adsorption, distribution, metabolism and elimination (ADME) determine the bioaccumulation of hydrophobic organic compounds in fish and mammals. Mechanistic models of bioaccumulation explicitly consider these ADME processes but there is a lack of appropriate data as model input parameters, particularly for compounds that do not partition simply to tissue lipid.There are a variety of in-vitro systems available for estimating ADME properties. Here we explore the applicability of these in-vitro assays for estimating ADME input parameters for bioaccumulation models. Caco2 cell lines, the parallel artificial membrane permeability assay and related assays can only be applied for rather hydrophilic chemicals and need adaptation for hydrophobic chemicals. Passive absorption and elimination through fish gills can be described by a modified parallel artificial membrane permeability assay that is tuned to hydrophobic chemicals, for which resistance of overall permeability is aqueous boundary layer controlled. In-vivo metabolism rate can be estimated using a variety of in-vitro systems including hepatocytes and microsomal or S9 fractions of fish liver in combination with a physiologically based prediction model. The reviewed in-vitro approaches are promising tools for identification and prioritization of chemicals in bioaccumulation assessment. Their application in regulatory assessment schemes is relatively new and will require continued validation to fully elucidate their potential advantages and limitations.

SS11-6

The EUROECOTOX EU-7th-framework project - identification of gaps and limiting steps for reduction, replacement and/or refinement of animal experiments used in environmental risk assessment

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Animal experiments play an integral role in current environmental risk assessment for the registration of chemicals, pesticides, biocides, pharmaceuticals and testing of whole effluents. As with risk assessment in human toxicology, there is a strong societal demand to replace, reduce or refine the animal experiments performed in order to protect the environment. However, efforts to develop and validate alternatives for human risk assessment are relatively more advanced: international OECD guidelines based on alternative methods are already available for some endpoints (e.g. skin corrosion, eye irritation, phototoxicity, genotoxicity).EUROECOTOX (European Network for Alternative Testing Strategies in Ecotoxicology) is an European Union coordinated action project which aims at identifying the gaps and limiting steps for reduction, replacement and/or refinement of animal experiments used in environmental risk assessment. EUROECOTOX is performing an analysis of regulatory requirements for ecotoxicity testing, novel

strategies and approaches to reduce vertebrate testing (fish, birds, amphibians), potential bottlenecks for validating new methods and measures to accelerate development and validation of alternatives. Recently, EUROECOTOX has identified the lack of OECD guidelines, the need to consider criteria of validation (reliability and relevance) early in development, (financial) support for validation of new approaches and limited access to existing animal test data for the scientific community as one of the major limiting issues.A detailed analysis and statistics of currently available alternative approaches and limitations will be given, supplemented by experimental findings from inside the consortium und published studies. The audience is invited to subscribe to the EUROECOTOX network and to discuss measures for advancing the development and acceptance of alternative methods in environmental risk assessment (http://www.euroecotox.eu).

SS12-1
Introduction and resume of approaches and criteria to establish critical loads and related concepts for selected environmental impact categories and their applicability to a global chemical footprint
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The presentation will give an introduction to how the critical load concept, and related concepts to characterise allowable pollution loads, have been developed, and what considerations have been necessary to arrive at agreed levels. The applicabililty of such concepts to th global chemicla footprint will be briefly discussed.

SS12-10
Taking action on high-concern chemicals - The NGO perspective
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The ever-increasing use of chemicals in virtually every product used in modern life has enabled improvements in society globally. However, the widespread use of chemicals has introduced actual and potential problems to man and the environment on a scale that is virtually impossible to estimate. The rise of different forms of cancer, decrease in reproductivity, increased obesity, emergence of behavioral disorders among children etc. are only some examples of the problems increasinlgy associated with exposure to high concern chemicals.In parallel with improving the tools to measure these effects, and reaching agreement on how to classify the chemicals causing these effects, one must, from an environmental health perspective focus on what to do to prevent damage from occurring in the first place.Our focus is not so much the cut-off values that can potentially be reached before irreversible damage is achieved.Rather, the question also has to be asked: What negative effect of chemicals exposure is accepted? On an individual as well as societal level. Not only how far can we go? But how far are we, as a society willing to go?

SS12-2
Planetary boundaries and chemical pollution
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The planetary boundaries concept includes 9 boundaries for different global environmental variables within which humanity can operate safely: climate change, ocean acidification, stratospheric ozone depletion, nitrogen and phosphorus biogeochemical cycles, global freshwater use, land use, biodiversity loss, atmospheric aerosol loading and chemical pollution [1]. Seven of these boundaries could be quantified. However, boundaries for aerosol loading and chemical pollution were not possible to quantify. Possible criteria for quantifying the global chemical pollution boundary are emissions, concentrations or effects on ecosystems of persistent organic pollutants (POPs), endocrine disruptors, plastics, heavy metals and nuclear waste. Due to the large number of chemicals in commerce, it is impossible to measure all possible chemicals in the environment. Another major stumbling block is lack of understanding of the effects of chemical mixtures on organisms, ecosystems and Earth system functions. Two complementary approaches were identified as possible ways to define a planetary boundary for chemical pollution: 1) focus on POPs with global distributions and 2) identify unacceptable, long-term, large-scale effects of chemical pollution on living organisms [1]. Data for only a few chemicals with POPs characteristics are available, for example, mercury, DDT and PCBs. In the second case, boundaries focussing on effects could be based on impacts on reproduction, immune systems and neurobehavior, particularly in sensitive species at sensitive life stages. However, it is currently difficult to link many chemicals to specific effects due to lack of toxicity data. One example of how this approach might work was the observed increase in neurodevelopmental disorders (autism, ADHD etc.) seen in children. A large number of chemicals are known to be neurotoxic in experimental animals and in humans, and five (methyl mercury, arsenic, lead, PCBs, toluene) are known to be toxic to human neurodevelopment. Thus, widespread exposure to low concentrations of many chemicals with known or suspected neurotoxic effects may have created a silent pandemic of neurodevelopmental disorders in children on a global scale [2].[1] Rockström J et al. 2009. A safe operating space for humanity. Nature 461:472-475.[2] Grandjean P, Landrigan PJ. 2006. Developmental neurotoxicity of industrial chemicals. Lancet 386:2167-2178.

SS12-3
Emissions of chemicals - important or misleading for a planetary boundary of chemical pollution?
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The concept of planetary boundaries is designed to indicate possible serious anthropogenic disturbances of the function of the Earth system, and chemical pollution might be an important contributor to the overall burden of humans on the planet. The concept as such (Rockstrom, Steffen et al. 2009) want to define a "safe operating space for humanity"in order to maintain the planetary conditions within ranges that we know are safe. The primary example is global warming where several studies point to the possibility of defining a range of radiative forcing within which climate will still be without unexpected serious changes related to nonlinear responses of the system.Is this approach applicable to all forms of chemical pollution? Chemicals may impact through specific mechanisms at sensitive points of the earth system, such as e.g. CFCs on the ozone layer, or due to other effects, more or less specific, on biota. In that case, also possible combined effects of many small contributions must be considered. In the case of impacts on biota, there is also links to another - already defined - planetary boundary for biodiversity that need consideration.However, before chemicals can have any effect they need to be emitted, but traditionally the scientific focus has been on fate (environmental chemistry) or effects (environmental toxicology) possibly due to the simple fact that persistence early was found to be a key property of substances identified as problematic - the silent spring paradigm regarded highly persistent, biomagnifying and very toxic substances - the PBT-approach to chemical regulation has since then dominated the scene.Present chemical regulation in Europe (REACH) focus on single substances and chemical products and does consequently only touch lightly upon the range of chemicals occurring in the societal stocks of products with longevities spanning from months to decades. These products include ordinary consumer goods like textiles, kitchenware and food pckaging and products made to last for long time like furniture, carpets, flooring, wallpapers and other building materials.The presentation will attempt at a discussion of the inclusion or omission of emission as part of a planetary boundary for chemical pollution in the light of what is known regarding other kinds of emissions of importance for the concept of planetary boundaries and on the possibility to use models to estimate emissions from materials.

SS12-4
Do we have the concepts and tools to estimate the global exposure to anthropogenic chemicals?
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Chemical pollution is not only a combination of many local or regional cases of environmental contamination, but has also truly global aspects. These include that there are chemicals with the potential to be distributed around the globe, in particular Persistent Organic Pollutants (POPs), and that there is a global trade in chemicals and chemical wastes that leads to widespread distribution even of chemicals without POP properties. This global nature of the chemical pollution problem is illustrated by the fact that the new UNEP Global Environmental Outlook (GEO-5 [1]) will, for the first time, include a chapter on chemicals and wastes. The Planetary Boundaries concept is important because it makes it possible to explicitly address these global aspects of chemical pollution. As an essential element of an assessment of chemical pollution at the global level, we need to quantify the total global emissions of a wide range of chemicals and the resulting global exposure to these chemicals. In this talk, it will be demonstrated that the combination of educated guesses of emission rates and multi-media mass balance models provides a means to reliably estimate the global exposure to anthropogenic chemicals. Global exposure includes levels of chemicals in various environmental media such as air, water, soil, and vegetation and in various regions of the globe, reaching from urban to pristine. For several types of chemicals, model results based on estimates of global emissions show good agreement with field data. It is concluded that today the tools that are needed to estimate global exposure to anthropogenic chemicals are available. Because of large data gaps in emissions and chemical property data, these estimates of global exposure to chemicals are still surrounded by uncertainties, but nevertheless a relatively clear picture of the global burden of anthropogenic chemicals can be established. Even on the global scale with maximum dilution by large volumes of water and air, average concentrations in ocean water in the pg/L range are observed for single POP-type chemicals when current emission rates are used. The combination of all relevant chemicals in commerce makes the total concentration of chemicals available for uptake by humans and wildlife even higher. [1] Global Environmental Outlook, to be published by the United Nations Environment Programme in May 2012: http://www.unep.org/geo/.

SS12-5
Planetary boundaries: a suitable concept for chemical pollution?
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The concept of planetary boundaries has been introduced by Rockström et al. in a publication in Nature in 2009. They are defined as 'scientifically informed values of the control variable established by societies at a 'safe' distance from dangerous thresholds'. That is, planetary boundaries indicate critical thresholds that must not be exceeded, in order to avoid catastrophic environmental impacts. Rockström and his colleagues suggested specific planetary boundaries for climate change, ocean acidification, stratospheric

ozone depletion, land and freshwater use, biodiversity loss and interferences with phosphorus and nitrogen cycling. They also suggest setting a planetary boundary for chemical pollution, which has not yet been quantified. This presentation will therefore explore whether it is indeed possible and sensible to apply the concept of planetary boundaries to toxic chemicals in the environment.Limitations of and challenges for the planetary boundary concept are not only related to emission, fate and exposure assessment (which were discussed in the previous talks). To a large extent they are also connected to the ecotoxicology of chemicals and include (a) the need to account for the ecological effects of complex multi-component mixtures, (b) the ignorance of local conditions and local effects, and (c) the enormous differences in the ecotoxicological profiles of the myriads of chemicals used in society, which, together with the ever-changing chemical use patterns, make any boundary a constantly moving target.We will explore those issues and their consequences for the concept of planetary boundaries for chemical pollution. To this aim we will use two groups of environmentally important chemical groups as cases in point: (1) a particular group of pesticides, so-called photosystem-II inhibiting herbicides, (2) unspecifically acting industrial chemicals (so-called baseline toxicants).

SS12-6

Fate-based categorization of chemicals and GIS Fate Model for the planetary boundaries concept

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It may be difficult to set up single criteria for the Planetary Boundary of chemical pollution, because chemicals have different mechanisms of effects on organisms including humans and different modes of emissions to the environment. First of all, it is necessary to consider simultaneously both the categorization of chemicals based on characteristics and the prioritization of the categorized groups. Categorization of chemicals might be decided as matrix of their characteristics such as environmental fate, toxicities, and other relevant information. As for environmental fate, though POPs (Persistent Organic Pollutants) and POPs-like chemicals should be important, chemicals which are not POPs-like but having temporal-spatial local peak should also be considered in terms of the Planetary Boundaries discussion. For example, if some chemicals locally affect some microorganisms on their reproduction and this impact affect other plants or animals, this issue is no longer a local problem. This means we should consider not only environmental fate of chemicals but also these ultimate impact to the planet. Data gaps and amount of available information, for especially new chemicals, also should be important when we select an appropriate method of the chemical risk assessment. Each category of chemicals should have each appropriate method. Although the simplified models like SimpleBox, CalTox, and ChemRange are useful for wide range of chemicals in faster and with less resource, the models are also indispensable that aims to reproduce the chemical fate processes in more details. In this presentation, we introduce our research about the detailed multimedia fate models developed for pesticides (PeCHREM/G-CIEMS) and the global model developed for POPs (FATE). As for the PeCHREM/G-CIEMS, in order to accurately predict the temporal-spatial variations of many pesticides in the environment, we constructed the prediction methods using general statistics of pesticide use, geographical, meteorological, hydrological database. As for the FATE, two issues were important that are to solve the temporally-spatially resolved emission inventory and to solve the general circulation of the atmosphere and the ocean. We also show the possible way to contribute these models to the Planetary Boundaries concept.

SS12-7

A planetary boundary for chemical pollution

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Johan Rockström and co-authors (2009) wrote a persuasive argument that human activity is exceeding the planet's biophysical boundaries. Rockström et al. defined 10 boundaries that we need to live within, or alternatively, face a radically different future. Chemical pollution was one of the 10 boundaries discussed, but the authors were unable to define the actual boundary. Unlike the other 9 boundaries, defining a boundary for chemical pollution will be challenging because the boundary for chemical pollution does not have a single variable, metric or indicator. We argue that a planetary boundary for chemical pollution is necessary to address the local to global nature of chemical release and transport, and the myriad and often subtle adverse effects that are arising from single and multiple chemical exposures. Furthermore, populations are increasingly vulnerable to adverse effects from chemical pollution as a result of experiencing multiple stresses, i.e., the global system abutting against other planetary boundaries. We suggest starting the planetary boundary discussion by exploring the similarity with the "critical load"concept. A critical load is defined as the highest load that can be added to a system without causing a specified adverse effect in a sensitive population or ecosystem. A critical load is estimated by first choosing a sensitive toxicity endpoint in a sensitive organism, population or ecosystem, from which the corresponding emission rate is back-calculated. For the planetary boundary, the calculation should be done on a spatially resolved global scale with attention paid to ecologically vulnerable systems. Into the calculation we need to specify chemical emissions as tied to annual chemical production and inventory. Environmental chemical persistence, in addition to chemical persistence brought on by human stockpiling, must also be addressed.Perhaps most challenging, we need to consider multiple chemical emissions rather than take a chemical-by-chemical approach. As a first step, we suggest calculating single-chemical critical loads or planetary boundaries for the few, relatively well understood high production volume chemicals for which we can gather sufficient information on physical-chemical properties, emission rates, inventory, environmental fate, exposure and toxicity. Considering lower production volume chemicals may be unnecessary.

SS12-8

Assessing the principle of a planetary boundary for chemicals used in personal care products

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As the per capita GDP of the world's population continues to grow, particularly in developing countries, such as Brazil, Russia, India, and China, which are expected to be the four biggest economies by 2050, it is likely that the use of chemicals in commerce that is associated with modern societies will significantly increase over the next several decades. It is thus important that governance approaches are sufficient to inform risk management discussions for reducing environmental impacts related to economic growth, and are based on rigorous scientific assessment of risks. Johan Rockström and colleagues (2009) proposed nine planetary boundaries of safe operating space for humanity. The authors suggest that these boundaries might stimulate a discussion for novel and adaptive governance approaches at the global, regional, and local levels. Included in the nine planetary boundaries is a boundary proposed for chemical pollution, which Rockström and colleagues do not quantitatively define. A number of questions thus follow from the proposal to define a safe operating space for humanity with respect to quantifying a planetary boundary for chemical pollution. For instance, what is meant by safe operating space and what is the relationship with other concepts, such as current approaches to environmental risk assessment, life-cycle assessment, ecosystem services or source to outcome pathways? In an effort to ensure that the use of chemicals in society associated with economic growth do not negatively impact the environment, we consider here the opportunities that the principle of a planetary boundary for chemical pollution offers, by exploring the utility of existing science-based assessment methods to inform decision making, assessing the role of technological innovation in relation to economic growth as well as issues of spatial and temporal scales, and how these parameters influence our ability to quantify a planetary boundary for chemical pollution.

SS12-9

Sound Chemicals Management - The policy perspective

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The aim of the Strategic Approach to International Chemicals Management is to achieve, by 2020, that throughout their life cycle chemicals are used and produced in ways that lead to the minimizationof significant adverse effects on human health and the environment. While significant progress has been made in some parts of the world, progress is sometimes uneven and that not enough is being done to reach the 2020 WSSD chemicals goal.The unsound management of chemicals can lead to the contamination of air, water and soil, resulting in increased human exposure and associated health risks. Sound chemicals management through the life cycle should be one of the key factors underpinning efforts to achieve poverty eradication and a green economy. The intention of this paper is to give state of art and to draw the attention to some areas where further progress needs to be made.

EC01 - Advances in passive sampling and dosing techniques

EC01A-1

PAH, PCB and OCP concentrations in the sediments, local mussels, transplanted mussels and passive samplers in the Istanbul Strait and Marmara Sea, Turkey

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Lipophylic organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and persistent organic pollutants (POPs) are accumulated in sediments and in organisms. The accumulated pollutants especially in fish and mussels may lead to serious human health hazards. In this study, the surface sediments and mussels collected from five sites of the Istanbul Strait and Marmara Sea as well as the transplanted mussels and deployed passive samplers were analyzed by using a high resolution mass spectrometer coupled with an gas chromatography to determine the concentrations of PAHs, polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCP). Istanbul Strait is a part of the Turkish Strait system connecting the Black Sea to the Medditerranean and one of the busiest waterways in the world. Maximum sediment T-PAH concentration (6431 ng/g) was measured at the sampling site representing the shipyard area, whereas the highest mussel T-PAH concentration (201 ng/g) was determined at a site situated at the middle part of the Strait at the mouth of a creek. The lower amounts of T-PAH concentrations in mussels were detected compared to passive samplers. The maximum sediment (25602 ng/g) and local mussel (881 ng/g) T-PCB concentrations were measured at the middle part of the Strait. In general, the differences in the T-PCB concentrations of the measured matrices were not significant. This shows that the PCB compounds could not be metabolized as the PAH compounds by the mussels. The accumulation of PCBs by the BR sorbents were not efficient as in case of PAHs. When SPMDs and BR sorbents were compared, the accumulation of PCB congeners in SPMDs were found much higher compared to the BR sorbents. OCP concentrations were only measured in sediments and mussels. The highest concentrations were measured at shipyard area. The most dominant OCP compounds were HCH and DDT derivatives. DDT concentrations in sediments were higher compared to the concentrations in mussels.

EC01A-2

Equilibrium sampling of environmental pollutants in Baltic Sea sediment along a transect in the Stockholm Archipelago

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The determination of the fugacity, freely dissolved concentration (C_{free}) or chemical activity of environmental pollutants in sediment is important since sediment is the dominant exposure medium for bottom-dwelling organisms and therefore is particularly relevant in a bioaccumulation context. Further, sediment represents the major reservoir for numerous hydrophobic organic chemicals (HOCs) in the aquatic environment, and the chemical activity in the sediment can govern the chemical activity in the water column.

To measure C_{free} of HOCs such as polychlorinated biphenyls (PCBs), a polymer is brought into contact with the sediment and analysed after equilibrium between the two phases is established. Here we used a coated glass jar with a very thin coating of polydimethylsiloxane (PDMS) on the vertical walls. The method makes use of three different coating thicknesses (2 µm, 4 µm and 8 µm), which allows equilibrium between sample and PDMS to be confirmed for each sample and analyte (QA/QC), while at the same time verifying the absence of sampling artefacts.

In this study, we investigated C_{free} of PCBs in sediment samples from the Baltic Sea, collected along a gradient from central Stockholm towards the middle of the Stockholm archipelago. We confirmed the applicability of the coated glass jar method in the open Baltic Sea. The results showed a decrease in C_{free} with increasing distance from the urban center. This indicates that the Stockholm waters continue to be a source of PCBs to the Baltic Sea close to four decades after the PCB ban in the 1970ies. The gradient could be caused by either ongoing releases to water or PCB residues in sediment from past emissions.

Further, we calculated PCB concentrations in biota lipids (C_{Lipid}) and K_d values that express the sorption strength of PCBs to the different sediments. These data were compared to values from the literature and the spatial distribution of C_{free} and *K*d was examined. In summary, passive equilibrium sampling using coated glass jars was shown to be a convenient, accurate and sensitive technique to determine C_{free} of PCBs in Baltic Sea sediments.

EC01A-3

Effects of flow velocity and calibration conditions on a passive sampler for perfluorinated alkyl carboxylates and sulfonates in water

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Perfluorinated chemicals (PFCs) are emerging environmental contaminants with a global distribution. Due to the moderate water solubility of some PFCs, the majority of the environmental burden is in the water phase. Passive sampling provides a low cost and time integrative sampling approach that has already proven useful for a broad range of environmental contaminants. A newly developed and validated Polar Organic Chemical Integrative Sampler (POCIS) with a weak anion exchange sorbent has shown potential as a passive sampler for PFCs in water. However more work was required to further validate the sampler. The aim of this work was to evaluate the influence of water flow rate and calibration conditions on the uptake of PFCs into POCIS sampler. Uptake kinetics and sampling rates for PFCs did not vary significantly with flow velocity. Sampling rates derived (0.08 - 0.28 L day-1) are comparable to sampling rates determined in a previous study under different conditions. A passive sampler for PFC and similar compounds could help elucidate potential aquatic exposure routes to PFCs.

EC01A-4

Occurrence and fate of brominated and organophosphorus flame retardants in river water

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The aim of this study was to evaluate the occurrence and fate of a wide range of flame retardants (FR) in river waters using a novel passive sampler and active sampling. Target compounds included PBDEs (BDE 28, 47, 99, 100, 153, 154, 183 and 209), new brominated flame retardants (hexabromobenzene - HBB, pentabromoethyl benzene - PBEB, pentabromotoluene - PBT, 2,3-dibromopropyl 2,4,6 tribromophenyl ether - DPTE, 2-ethylhexyl 2,3,4,5 tetrabromobenzoate - EHTBB, Bis(2-ethyl-1-hexyl)tetrabromo phtalate - BEHTBP, 1,2 bis (2,4,6 tribromophenoxy)ethane - BTBPE and decabromodiphenyl ethane - DBDPE) and organophosphorus flame retardants (tris(2-choroethyl) phosphate - TCEP, tris(2-chloro-1-methylethyl) phosphate - TCPP, tris[2-chloro-1-(chloromethyl)ethyl] phosphate - TDCP and triphenylphosphate - TPhP). Analysis was performed by gas chromatography coupled to mass spectrometry (GC-MS). As a first step, a passive sampler design based on a ceramic dosimeter and HLB as receive phase was tested and calibrated in laboratory conditions to determine uptake kinetics of a set of six flame retardants. The sampling rate obtained for TCPP, TCEP, TDCP and TPhP were 2.4, 2.9, 2.3 and 2.1 mL day-1, respectively, while lower values were found for the rest of FR, such as for HBB and PBEB (0.4 and 0.6 mL day-1, respectively). In a second step, a monitoring study was performed along the River Aire, in Yorkshire, Northeastern England using grab samples and the passive sampler. In this latter case, unfiltered waters were extracted by means of solid phase extraction (SPE), through OASIS HLB cartridges. In grab samples, TCPP was the most abundant compound, probably due its increased local usage and punctual sources, being detected in all samples, ranging from 0.11 to 26 µg L-1 (average 8.3 µg L-1). TCEP, TDCP and TPhP were detected in most of the sampling points ranging from 0.12 to 0.32 µg L-1, 0.06 to 0.15 µg L-1 and 0.006 to 0.02 µg L-1, respectively. From the brominated flame retardants, the BDE 209 was frequenty detected, ranging from 0.02 to 0.29 µg L-1. When comparing the performance of active and passive sampling, a good correlation was observed for organophosphorus flame retardants after a 3-week deployment period, while BDE 209 was only detected in the grab samples. Overall, the developed passive sampler proved to be efficient for the monitoring of organophosphorus flame retardants, a family of compounds which is characterized for their widespread occurrence and high toxiciy.

EC01A-5

Porewater profiles of As, Se, Fe, Mn, V and P in spiked marine sediment measured using DGT and DET techniques

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Arsenic and selenium are anionic contaminants, released into aquatic environments from the mineralisation of naturally-rich ores and industrial discharges. A diffusive gradients in thin films (DGT) technique to measure pore water profiles of As, Se, Fe, Mn, V and P in spiked marine sediment is described. The study evaluates the performance of DET and two DGTs (precipitated ferrihydrite and titanium dioxide (Metsorb[TRADEMARK]) binding layers) in sediments that were sieved (<1 mm), aged for 8 months. The mangrove sediments were then spiked at 60 mm depth with either As(III) and Se(IV), or As(V) and Se(VI) 48-h prior to probe deployment. DET profiles identified a Fe/Mn redox boundary at 40 mm below the sediment water interface, which correlated with the DET-P maxima. Both ferrihydrite and Metsorb[TRADEMARK] DGTs successfully bound As(III) and As(V) (as total As), Se(IV), and natural levels of V(V) and PO_4^{3-} from marine sediment porewaters (Se(VI) was not DGT labile). There was more

DGT-labile Se, As and V in the sub-oxic and oxic regions of the sediment and overlying waters. Differences in DGT and DET profiles are attributed to DET measuring total dissolved solutes whereas only the oxyanions were DGT labile, demonstrating the benefits of dual DET and DGT deployments

EC01A-6

Polar (un)charged micropollutants on SPE materials - which factors control the adsorption?

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Adsorption of organic solutes from the water phase onto solid matter is a crucial process in the environment, water purification and in analytical science. The ability to adsorb to and absorb into a material dictates the fate of solutes in the environment. Many of these solutes are polar or even charged. Adsorption of these compounds to SPE materials is a promising way to help detecting them, as concentrations are often still too low for MS and preconcentration can overcome this problem. Many materials have been employed for this purpose, but little is known about the exact sorption mechanism of these polar compounds. The role of functional groups of these solutes and the SPE materials is still ambiguous. How do they interact with each other and what is the part of water in the adsorption process? Apart from that, the effects of other compounds, such as inorganic salts, on the sorption behaviour is not yet well-understood. This knowledge can also be used for the removal of these compounds from drinking water. Often they pass through the treatment plant, as the current treatment methods cannot remove them effectively.
Aim of the research is to get a better insight into the influence of various functional groups of selected chemicals and SPE materials on the sorption to be able to take a grounded decision which SPE material should be used. In case of charged organic compounds, additionally the impact of competing inorganic electrolytes was monitored. We decided to use OASIS polymers as SPE material. These polymers carry polar moieties, hydrophilic parts as well as charged groups, which should allow the adsorption of the target compounds.
The results of this research indicate that especially apolar functionalities have a great impact on the sorption, whether the compound is charged or not. The more pronounced the apolar moiety is, the better the compound can adsorb. However, the results show that there is no direct correlation between solubility and adsorption behaviour. Furthermore, it emerged that the conditions of the aqueous phase, such as salt concentration, influence the sorption behaviour of charged molecules dramatically. The higher the salt concentration is, the lower the adsorption of the charged compounds. Apart from concentration also the type of ion is important.

EC01B-1

Stimulation and Inhibition of bacterial growth by caffeine dependent on antibiotics and silver nanoparticles - a ternary toxicity study using a microfluid segment technique

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A droplet-based micro segmented flow technique for the fast generation of three dimensional concentration spaces for the screening of toxic effects of selected antibiotic substance and silver nanoparticles on the toxicity and activation of bacterial growth by caffeine at the nanoliter scale was introduced. Up to about 1200 well separated fluidic compartments containing 216 different combinations of concentrations were realized in a single experimental run. To evaluate the toxicity of the ternary mixtures a time resolved miniaturized optical double endpoint detection unit using a microflow-through fluorimeter and a three channel microflow-through photometer were used for the simultaneous analysis of changes on the endogenous cellular fluorescence signal and on the cell density of E. coli cultivated inside 500 nL microfluid segments. As a result, a complex response pattern was discovered including synergistic and compensatory effects as well as strong non-linear combination effects, concentration dependent stimulation and the formation of activity summits on isobolographic maps. The results reflect a complex response of growing bacterial cultures depending on the combined effectors.
A strong caffeine induced enhancement of bacterial growth was found at sublethal chloramphenicol and sublethal silver nanoparticle concentrations. The reliability of the method was proven through the high redundancy of fluidic experiments. The results indicate the importance of multi-parameter investigations for toxicological studies and prove the potential of the microsegmented flow technique for analyzing combined effects.

EC01B-2

Applying novel passive dosing systems for the control of chemical and drought stress to a terrestrial invertebrate

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Two passive dosing systems were used in this study to control (**1**) chemical stress and (**2**) combined chemical and drought stress in toxicity tests with the terrestrial springtail *Folsomia candida*. *F. candida* lives in the interstitial air in the top soil and is very sensitive to low humidity. The test compounds were the PAHs naphthalene, phenanthrene and pyrene. The aims of the studies were (**1**) to link lethality of naphthalene, phenanthrene, pyrene and their mixtures to (sum) chemical activity and (**2**) to determine springtail lethality in a two-factor experiment with both chemical and drought stress. Chemical activity is a multimedia parameter that expresses the energetic state of the contaminant. Passive dosing from PAH loaded silicone elastomer was used to control defined chemical activities of the test compounds in glass vials. To start the 7-days test, 10 springtails were transferred to each loaded vial. *F. candida* could move freely on the loaded silicone, resulting in exposure through direct contact and headspace. In the test with combined chemical and drought stress, the chemical exposure was controlled by passive dosing, as described above. Passive dosing vials were covered with a net and placed inside a closed glass jar with aqueous saline solution in the bottom which controlled the humidity within the entire jar. In this way, *F. candida* was exposed to controlled chemical stress from below, while the drought stress was controlled from above. The PAH exposure parameter was in all cases chemical activity (unitless, [0-1]), and the humidity was expressed as relative humidity, that is also known as water activity. The two passive dosing systems were simple and practical to use, and the systems worked well with high survival in controls and low variation in lethality within treatments. Springtail lethality caused by naphthalene, phenanthrene and pyrene was successfully linked to chemical activity. Springtail lethality caused by four PAH mixtures (each in three dilutions) was plotted as a function of sum chemical activities, and the toxicity data of all tested mixtures was successfully fitted to one chemical activity response relationship. The lethal chemical activities (La-50) of the individual PAHs and the mixtures were all well within the expected range of 0.01-0.1 for baseline toxicity. The results from the combined stressors experiment showed stress synergy in springtail lethality between the chemical and drought stress.

EC01B-3

Passive dosing under the microscope reveals that microorganisms enhance the mass transfer of hydrophobic organic chemicals

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The transport of hydrophobic organic chemicals (HOCs) is often rate limited by their diffusive mass transfer through stagnant boundary layers (SBLs). This then results in gradients on the microscale, which are crucial for the diffusive transport of HOCs and for organisms living in such boundary layers. To study how microorganisms react to such gradients, and whether they can alter the mass transfer of HOCs through SBLs, a new experimental setup was developed. In this setup, microgradients of HOCs could be precisely controlled and quantified. At the same time microscopic observation of organisms exposed to the gradients could be performed. Passive dosing was employed on microscope slides to produce gradients of polyaromatic hydrocarbons (PAHs) by integrating silicone O-rings into the Dunn Chemotaxis chamber. An outer clean ring thereby served as a sink and an inner PAH-loaded ring as a source. The mass transfer of the PAHs from source to sink by diffusion through protozoa medium was then quantified, and compared to that in the presence of the ciliate Tetrahymena pyriformis. In the presence of T. pyriformis the mass transfer was enhanced, increasing with the hydrophobicity of the PAHs. This enhancement was nearly hundred-fold for benzo[a]pyrene, the most hydrophobic compound tested. Fluorescence video imaging microscopy showed that this enhancement was based on a mechanism where protozoa acted as a transport vector of PAHs via their diffusive uptake and release. Such a transport mechanisms has, to our knowledge, not yet been described.

EC01B-4

Comparison of two monitoring strategies for organic pollutants in seawater: passive sampler devices vs transplanted clams

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The concentration of organic pollutants in seawater shows a great spatial and temporal variability as a result of a combination of natural and anthropogenic effects. In this study we have characterized simultaneously the bioaccumulation in transplanted clams and two passive sampling devices (semipermeable membrane device, SPMD, and continuous flow integrative sampler, CFIS). The combination of these monitoring techniques such as water, biota and passive samplers with the analysis of water concentrations allows a comprehensive knowledge of the environmental status and to assess the representativity of these integrative sampling methods. The efficiency of these two passive samplers and the determination of the bioaccumulation of organic pollutants in transplanted clams were tested in the marine environment in spring and autumn comparing the obtained integrative concentration with the real water one. The study was performed in four selected sampling points in Mar Menor Lagoon (SE of Spain), which is subjected to direct and indirect discharges of organic pollutants. El Albujón Wadi is the main surface watercourse that flows into this lagoon from Cartagena Field, which is one of the most relevant horticulture areas in Europe. The organic pollutants determined in clam were PAHs, PCBs and organochlorinated pesticides. In surface and marine waters PAHs, PCBs, triazines, organophosphorus and organochlorinated pesticides were analysed by stir bar sorptive extraction and thermal desorption coupled to capillary gas chromatography-mass spectrometry (SBSE-TD/GC/MS). Significant daily, weekly and seasonally differences were observed in the input of organic pollutants. Chlorpyrifos ranged from 0 to 12 ng/L in spring and from 3 to 6000 ng/L in autumn. Duplicate and triplicate of CFIS and SPMD were studied in several sampling points in

order to study the repeatability (RSD<20%) of passive samplers. Chlorpyriphos was detected in spring in all cases, both in SPMD (4.8-130 ng/L), showing a great variability, and CFIS (4.68-17.25 ng/L) this insecticide only detected with CFIS (2-18 ng/L) in autumn. An increase of concentrations in clams for pesticides and PCBs were observed after a month of exposition in the mouth of El Albujón watercourse. In general both in Spring and Autmn the intermediate exposure time was too short to reach similar pollutants levels found in CFIS or SPMD.

EC01B-5

A passive sampling method for the estimation of concentrations in pore water and accessible concentrations of polycyclic aromatic hydrocarbons in contaminated sediments

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For the environmental risk assessment of contaminated sediments it is important to know both the equilibrium concentrations in the pore water (C_w) and the accessible concentration in the sediment phase (C_{as}). C_w is highly relevant for uptake by organisms and transport, and the accessible concentrations in sediment are important for distinguishing between the contaminant fractions that may be subject to bioaccumulation and bioremediation on one hand, and fractions that are strongly bound to resistant sediment constituents, such as soot and coal, on the other. Traditional methods for determining accessible concentrations are extraction of sediment suspensions for a fixed period of time using gas purge or TENAX. Concentrations in the pore water traditionaly are measured in sediment suspensions by passive sampling (e.g. SPME, polyoxymethylene, low density polyethylene or silicone rubber), assuming that uptake by the passive sampler does not substantially lower the initial C_w.
We developed a silicone passive sampler based method for the simultaneous determination of C_w and C_{as}, by incubating sediment suspensions at increasing silicone /sediment phase ratios. This method allows for determining sediment-water sorption isotherms, because higher silicone masses cause a lowering of the concentrations in both the sediment and the pore water. Sediment samples from 3 different areas in the Netherlands were equilibrated with silicone samplers by shaking at 150 rpm for 28 days with 4-5 different sampler sediment ratios ranging from 0.03 to 1 at a suspension density of 0.1 g/mL. The results show that the accessible contaminant fraction follows linear sorption isotherms, and that only 10 25% of the total concentrations can be released from the sediment to the aqueous phase on the timescale of the experiments. In additional experiments, a strong increase in passive sampler uptake rates was observed at higher suspesion densities. The results are highly relevant for the risk assessment of contaminated sediments.

EC01B-6

Evaluation of an in-situ equilibrium sampling device for persistent organic pollutants in sediment pore water systems on the basis of solid phase microextraction (SPME)

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In this study, equilibrium passive sampling device is introduced that makes POP bioavailability in terms of freely dissolved aqueous concentrations assessable on site, i.e. in the sediments. The developed in-situ sampling device has several advantages compared to in-vitro or solvent-based sample extractions that make it an ideal monitoring tool: (1) The ecologically relevant parameter 'in-situ bioavailability' is addressed instead of total sediment or pore water concentrations. (2) Due to short/medium equilibrium times, the temporal resolution of the measurements is suitable for analysis of both long-term trends and seasonal effects. (3) The device is of very solid construction and can be reused practically ad infinitum; only replacement of the disposable sampling materials contributes to its operational costs. (4) Sample treatment is reduced to a minimum which in turn reduces possible sources of sample manipulations, measurement errors and analysis costs. Both sampling and analysis procedures are thus simple, robust and cost-effective.
The device is already applicable in a multitude of aquatic environments, especially where currents are low and sediments are muddy and well-mixed e.g. by bioturbation. Examples for such environments are mud flats, harbor basins, river banks and lakes.

EC01C-1

Use of plants as passive samplers for volatile organic compounds (VOCs) in indoor environments

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Concerns about the potential build up of volatile organic compounds (VOCs) in indoor air have increased as energy conservation methods minimized the introduction of outdoor air. VOCs, including many with documented short- and long-term adverse health effects, are can enter indoor environments through internal (i.e. paints, paint strippers, fuels, cleaning supplies, pesticides, building materials, adhesives) and external sources (i.e. vapor intrusion from contaminated groundwater). Indoor air concentrations of VOCs vary widely, but concentrations of most VOCs are consistently higher indoors than outdoors. Typical approaches used to sample indoor air include evacuated canisters and sorbent tubes. The use of ornamental plants has been suggested as a simple, unobtrusive, aesthetically pleasing, and cost effective method for sampling and purifying indoor air. The waxy surface of the leaves has the potential to provide a good surface for the passive capture of VOCs. However, the efficiency and kinetics of capture has not been well characterized. To investigate the potential use of plants as indoor air VOC samplers, three types of studies were performed. The first consisted of monitoring air and plant concentrations over time after a controlled release of several VOCs into a residential building containing several plant species. The second study used a flow-through glass and stainless plant growth chamber to evaluate the relationship between air and plant leaf VOC concentrations. The third study used a headspace approach to measure equilibrium leaf-air partition coefficients. Good correlations between the leaf and air concentrations observed in the three different studies suggest that plant leaves can be used to monitor indoor air concentrations of VOCs.

EC01C-2

Calibration and field evaluation of Polar organic chemical integrative samplers (POCIS) for monitoring pharmaceuticals in hospital sewage water

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Pharmaceuticals have become an important issue in the last few years due to their continuous release in the environment. In this context, we made the MEDIFLUX study to estimate the contribution of hospital to the urban sewage pollution by pharmaceuticals. To go on, we decided to test Polar Organic Chemical Integrative Samplers (POCIS). Passive samplers allow the measurement of Time Weighted Average (TWA) concentrations, overcoming many shortcomings of the spot sampling techniques. The application of POCIS was studied using six compounds already selected as representative of the great families of pharmaceuticals used at the hospital (Atenolol, Prednisolone, Methylprednisolone, Sulfamethoxazole, Ofloxacin, Ketoprofen).
In a first step, POCIS were calibrated in tap water under laboratory conditions for the analytes of interest taking into account various relevant environmental conditions (temperature, flow rate..). POCIS were exposed under a constant agitation to tap water spiked with the selected pharmaceuticals, renewed every two days. Sampling rates were determined and compared. It appears that Rs increase significantly when flow rate increase between 10 to 25 cm.s-1 for all the compounds (except sulfamethoxazole). A slight difference of Rs between 15 and 25°C was observed for sulfamethoxazole, prednisolone, ketoprofen.
In a second step, the flow rate and temperature of waste water were recorded during a week on the selected hospital effluent. For the calibration, 20 L of wastewater were taken from hospital effluents and brought to the laboratory to carry out Rs measurement as for the previous measurement in tap water. Sewage water was changed every two days. The Rs values obtained were closed to Rs values in tap water.
As the laboratory calibration step shows the feasability of POCIS to sample sewage water, we plan to deploy POCIS in situ.
The step of calibration was the main part of the work and its completion was needed before the application of POCIS in situ. The follow-up of accumulation made it possible to estimate the field of linearity in order to choose the optimal length of POCIS implantation in situ.
The use of POCIS for the follow-up of contamination by organic pollutants is especially described for surface waters such as rivers, but very little studies report its use in wastewaters. This work gives encouraging results for the deployment of POCIS in sewage that could be a useful tool for pharmaceutical pollution management.

EC01C-3

Accumulation kinetics and sampling rates for 56 polar organic compounds, identification and validation of 5 PRCs

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POCIS (Polar Organic Chemical Integrative Sampler) is a new emerging tool for sampling polar organic micropollutants in water. There is still a need of research concerning its domain of validity (e.g., molecules sampled, type of water studied, optimal exposure duration) and its performances (molecules sampling rates, repeatability, accuracy of the evaluation of time-weighted average or TWA concentrations).
In order to obtain in situ TWA concentrations, POCIS needs to be calibrated in laboratory for each molecule of interest. We performed 3 different laboratory calibration experiments using a flow-through calibration system in order to evaluate the optimal exposure duration and to calculate the sampling rates for 56 polar organic contaminants, and to identify and validate performance reference compounds (PRCs). The calibration system was composed of 2 aquaria (50 L) containing i) spiked tap water (circa 3 µg/L) and non-spiked POCIS for the determination of accumulation kinetics, ii) non-spiked tap water and spiked POCIS for the determination of desorption kinetics and iii) spiked tap water and spiked POCIS for the validation of the potential PRCs. In all experiments, water temperature, pH, conductivity and dissolved organic carbon were controlled. Agitation in the aquaria was ensured via a submerged pump. The resulting flow velocity was 10±5 cm/s and was directed perpendicularly towards POCIS surface. Triplicate POCIS were analyzed at 0, 1, 3, 6 and 12 hours and at 1, 3, 7, 11, 14, 21 and 28 days.

We present results for the 56 studied molecules, including the duration of linear accumulation phase, sampling rates and possible performance reference compounds (PRCs). The accumulation phase was curvilinear for almost all molecules; so, we obtained the duration of optimal linear accumulation phase using t1/2 criteria. Concerning the desorption experiments, 5 compounds showed a good potential as PRC; they enable to decrease the effect of variable environmental conditions, so they can be used to obtain more reliable in situ TWA concentrations. We also checked isotropic exchange for these molecules comparing the exchange constant ke in accumulation and desorption experiments. At last, we validated the PRC strategy by calculating TWA concentrations from the third laboratory experiment mentioned above.

EC01C-4

Polar organic chemical integrative sampler (POCIS) calibration for steroid hormones and pharmaceuticals, and flow modelling in the calibration system

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Water pollution from emerging contaminants such as steroid hormones and pharmaceuticals is one of the important stakes of current environmental research. Pharmaceuticals that are most often encountered in surface water are antibiotics, non-steroidal anti-inflammatory drugs, anti-epileptic drugs and hypolipidemic agents with a proven toxicity on living organisms. Steroid hormones, either natural or synthetic, are also of major concern. These compounds are typically encountered in the aqueous environment at very low, but toxicologically relevant, concentrations (ng/L).
One of the main drawbacks of conventional grab sampling is the poor representativeness of the actual contamination. Passive sampling can be an alternative approach that provides a more accurate image of the contamination, while being easier to handle than large volume water samples. Polar Organic Chemical Integrative Sampler (POCIS) in its "Pharm"configuration contains Oasis HLB material enclosed within two polyethersulfone membranes. This design enables the POCIS to trap polar organic compounds. Another configuration with Nylon membranes has also been tested, allowing less polar compounds to reach the sequestration medium.
This work presents the results of a calibration with these two different POCIS designs, as well as desorption of target compounds from samplers. Various steroid hormones (Estrone, α-Estradiol, β-Estradiol, Ethinylestradiol, Estriol, Mestranol, Progesterone, Testosterone, Norethindrone, Levonorgestrel) and some widely used pharmaceuticals (Sulfamethoxazole, Erythromycin-H2O, Bezafibrate, Diclofenac, Carbamazepine) were studied.
In the literature, various calibration systems are used. In the present work, calibration of POCIS was carried out in a flow-through exposure system with stirring action provided by rotating blades. Computational Fluid Dynamics (CFD) modelling with Fluent software confirmed the homogeneity offered by this design and gave an estimation of the flow at the surface of the samplers.

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EC01C-5

The use of passive samplers to constrain distributed models for pesticide runoff simulations

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Passive samplers yield data on average concentration during their exposure period.Yet their application is most useful to cover periods where concentrations are strongly fluctuating and the information hidden in the average value should be decrypted. Pesticides are emitted by two main episodically active pathways: emissions from Wastewater Treatment Plants (WWTP), which are the result of the spilling of leftovers and the cleaning of spraying equipment during application periods, and surface-runoff from agricultural fields during precipitation events. Passive samplers are easy to install in greater numbers and can therefore be used to track critical source areas for pesticides.
The main issue relates to the quantitative nature of passive samplers. Sampling rates (R_s) can deviate rather largely under field conditions depending on the flow environment and the compound properties. Moreover, for the evaluation of peak concentrations the elimination rate (k_e) for the substances during non-polluted periods follwing the peak is equally important. Hence a robust determination of k_u (uptake rate) and k_e is the basis for the interpretation of plumes that have been monitored by a passive sampler.
This contribution shows uptake and elimination rates that have been determined in the laboratory and the field and how these parameters can be used to estimate peak concentrations during exposure periods of passive samplers. In combination with autosampler data from one site located near the basin outlet, contributions from low-flow and precitpitation events can be calculated with simple balancing of pesticide uptake by passive samplers during floodwaves for each site. The data have been used to refine sub-catchment emissions in a SWAT simulation.

EC01C-6

Calibration and exposure correction methods for the polar organic chemical integrative sampler (POCIS)

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The measurement of polar compounds of interest in complex environmental matrices and at trace concentrations represents a significant analytical challenge. In this regard, passive sampling devices may have much to offer the analytical process, for example providing a time-integrative sample, with low detection limits and in-situ extraction of analytes. The polar organic chemical integrative sampler (POCIS) has been shown to accumulate a wide range of such chemicals. However in contrast to hydrophobic passive samplers, results remain semi-quantitative. This is due to the lack of an uptake model and an exposure correction method which may adjust sampling rates for differences in environmental conditions during deployment. Thus the purpose of this study was to examine the variation due to environmental factors on uptake in POCIS and the possibility of correcting differences using performance reference compounds from hydrophobic passive samplers. Sampling rates for several hundred compounds from over 20 calibration studies published in the literature were critically examined. In addition, several flow through calibration experiments were conducted at a local sewage treatment works. Most published calibration studies describe static type experiments with either batch renewal or modelled depletion of exposure water. Whilst such experiments may be reasonably reproducible they are likely to be poorly replicated in the environment. Whilst the effect of environmental factors of POCIS sampling rates is generally somewhat smaller than that reported for hydrophobic samplers, water flow rates, temperature, fouling, are all reported to affect uptake. These effects were not uniform for all compounds examined. Additionally polar compounds may be more greatly affected by pH and salinity than hydrophobic ones. Calculated in situ sampling rates were in general lower than those for laboratory studies of similar compounds. Changes in sampling rates in co-deployed hydrophobic samplers were not easily related to those of POCIS, where both samplers were subjected to variable water flows. There remain gaps in our knowledge concerning polar samplers and the processes involved in chemical accumulation. In summary we need to address some of these basic questions before we can hope to use polar passive samplers in a truly quantitative way.

EC02 - Contaminant pathways, trends and biological effects in a warmer Arctic

EC02A-1

Influence of climate change on contaminant distribution and effects in Arctic marine food webs - Summary of the IPY project COPOL

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An overall aim of COPOL was to evaluate and quantify the seasonal and annual variation in bioaccumulation and effects of contaminants (organic contaminants and trace elements) in benthic and pelagic Arctic marine food webs. This was important in order to be able to separate the effect of a climate signal from other expected system variability. Two fjords on Svalbard with different climate conditions were selected as study sites: Kongsfjorden (Atlantic signal) and Liefdefjorden (Arctic signal).
In summary, the seasonal variation in the pelagic food web was greater than both between year and between fjord variations. This was found both for absolute concentrations from zooplankton to fish and birds, and for the average concentration increase per trophic level (food web biomagnification estimated by trophic magnification factors).
The high within year variability underlines the importance of coherent studies of specific time periods rather than pooling samples across a year. It also means that if studies should repeat annually, it is important to select one representative time period.
We assumed that the selected fjords in the site for time study would represent different climate scenario. However, there are several indicators that even the fjord on Svalbard's northern coastline was influenced by Atlantic waters. This was reflected in the pelagic food web that contained both Atlantic zoolankton and Atlantic cod. The benthic food web on the other hand, was a better reflection an Arctic site, and is like to better reflect the average conditions of the site.
The present study has documented large changes in bioaccumulation during the year, at a larger scale than between year or between fjords. It is therefore important to standardize future studies to identify the true source of the observed variation.

EC02A-2

Snow, contaminants and climate change

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The impact of a changing climate on contaminant fate in the physical environment of cold regions is likely to be amplified by the sensitivity of the cryosphere to relatively small temperature changes. It is not so much the change in temperature that will modify a contaminant's environmental behaviour, but the changes in the extent and duration of a seasonal snow and sea ice cover and in the nature of the snow melt. In order to anticipate the potential influence of climate change, we seek to improve the general understanding of contaminant-cryosphere interactions through a combination of laboratory experiments, field studies and computer simulations. Snow is subjected to controlled melting in a cold room laboratory and the frationated melt water is filtered and the sorbed and dissolved fractions analysed with appropriate trace analytical techniques. Elution curves for different contaminants of variable partitioning properties, for different types of snow and for different melting conditions are recorded. Field studies in a highly urbanised watershed involve the repeated sampling of river water throughout the snow melt season. Sorbed and dissolved fractions are analysed by trace analytical techniques. Elution curves of contaminants from a melting snow pack are predicted with a simple mass balance model that simulates the sequential melting of several horizontal snow layers and the resulting downward percolation of melt water. The model assumes equilibrium partitioning between the various snow pack phases. Laboratory experiments revealed at least five types of elution curves for organic contaminants from a melting snow pack. All types could be reproduced with, and thereby mechanistically explained by, the snow pack melt model. Elution curves in river water do not resemble those eluting from laboratory snow packs, except that water soluble contaminants tend to appear early during the melt period. Particle-bound contaminant concentrations in the river tend to peak sharply during snow melt, and correlate with river run-off rates. Concentration time profiles of particle bound contaminants in rivers during snow melt are less controlled by processes occurring within the snow pack, and depend more on factors that determine run-off rate and the mode of melt water ablation from the snow pack to the stream. Many of these factors are influenced by a changing climate.

EC02A-3
Climate change and Arctic marine mercury biogeochemistry - conclusions and research needs from the AMAP 2011 Arctic Mercury Science Assessment
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Recent studies have shown that climate change is already having significant impacts on many aspects of transport pathways, speciation and cycling of mercury (Hg) within Arctic ecosystems and ultimately biological exposure, including humans. For example, the extensive loss of sea-ice in the Arctic Ocean and the concurrent shift from greater proportions of perennial to annual ice have numerous effects including promoting increases in primary productivity, shifting foodweb structures, possibly altering methylation and demethylation rates, and influencing Hg distribution and transport across the ocean-sea-ice-atmosphere interface (i.e., bottom-up processes). In addition, changes in animal social behavior, such as habitat selection in association with changing sea-ice regimes by top predators such as beluga, can also affect dietary exposure to Hg (i.e., top-down processes). In this presentation, adapted from one chapter of the recent AMAP Mercury Science Assessment, we address these and other possible ramifications of climate variability on marine Hg cycling, processes and exposure in the Arctic.

EC02A-4
Local contaminant sources in the Arctic: volatile and non-volatile residues from combustion engines in surface soils from snow mobile tracks in the vicinity of Longyearbyen (Svalbard Norway)
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A summer field campaign was conducted in 2010 for investigating potential long-term effects on surface soil from fossil fuel burning.A total of 18soil samples and two surface snow samples were collected for the analysis of polycyclic aromatic hydrocarbons (PAHs) along frequently used snowmobile tracks in/out of Longyearbyen (78° 13' 0 N, 15° 37' 60 E). In addition, Benzene-Toluene-Xylene (BTX) components were measured in ambient air at he University Centre in Svalbard Laboratory facilities during spring and in autumn 2010. Polycyclic aromatic hydrocarbons (PAH) was determined in all 18 soil samples in the concentration range 21 to1883 ng/g dw (dry weight, SUM 15 PAH). All samples were characterized with pyrogenic PAH patterns.The highest concentrations were found in a sample from the former winter airfield close to the old Northern Light observatory. Only 30% of the 2007 concentrations were detected for Benzene in 2010 Longyearbyen air. This significant concentration reduction is assumed to be caused by the higher proportion of 4-stroke engine driven snowmobiles during the 2010 season.

EC02A-5
The impact of thawing permafrost on lakes of the Mackenzie Delta uplands, NT, Canada
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Total permafrost in the Northern Hemisphere currently occupies an area of 26 million km2, and by 2100, this area is expected to decrease by 19-35%. In the Mackenzie Delta, NWT, temperatures are projected to rise by 4 to 5°C in the next 50 years. Over the past 20 years, mercury and PCBs have been steadily rising in burbot from the Mackenzie River, which has prompted speculation on how the changing physical environment, such as thawing permafrost, might be affecting contaminant cycles in these thermokarst environments. We tested the hypothesis that the presence of retrogressive thaw slumps in the Mackenzie Delta Uplands (north of Inuvik, NT, Canada) is affecting nutrients (total and dissolved N and P), persistent organic pollutants, metal concentrations, and phytoplankton community assemblages in small tundra lakes. Dissolved organic carbon, total phosphorus, soluble reactive phosphorus, and total and methyl mercury were significantly lower in lakes with retrogressive thaw slumps than reference lakes, likely due to deeper water infiltration through clay-rich tundra soils. These results provide compelling evidence that thawing permafrost near lakes of the Mackenzie Delta uplands are not responsible for the rising trend in mercury concentrations of fish in the Mackenzie River.

EC02A-6
Burdens and inputs of perflourinated compounds in the Lomonosovfonna Ice Core, Svalbard (2009)
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In 2009, we drilled a 40-meter ice core representing ~1953 - 2008+ from Lomonosovfonna (78°49' 24.4' N; 17°25' 59.2'E), the highest elevation glacier on Svalbard (1250 m.a.s.l.). We sub-sampled the upper 21 meters of the core to identify inputs of 12 perfluorinated compounds (PFCs) delivered to the site by long-range atmospheric transport. Each sample had liquid volumes of 291 mL (average), and dating resolution averaged 2.4 years from 1976 - 2008. Five compounds were not detected in any segment of the core, including PFBA, PFPeA, PFHpA, PFBS, 6:2FTS. PFNA dominated the accumulated burden in the core, showing 29.4 pg cm-2, 81% more than the second highest, PFOA, 16.2 pg cm-2. It is thought that the PFNA and PFOA may be an atmospheric oxidation products of 8:2 FTOH which has been found to be the dominant gas-phase PFC in Europe. PFOA however, is considered to be the dominant PFC in the atmospheric particle phase in Europe; these results suggest that PFCs in both phases are reaching high-elevation ice on Svalbard. Other burdens include PFHxA and PFDA which were approximately equal (9.91 and 9.85 pg cm-2) and about one-third PFNA. No other PFC had a burden greater than 5.8 pg cm-2 (PFOS), including PFUnA, PFHpA and PFHxS. Net flux trends greater than 10 years duration appeared for PFNA, PFDA, and PFOS. The trend for PFNA begins in 1982 (0.388 pg cm-2 yr-1), increasing regularly until 1995 when it doubled to 1.25 pg cm-2 yr-1 by 1997, and reaching 5.5 pg cm-2 yr-1 by 2008. PFDA has the longest flux record beginning with 0.10 pg cm-2 yr-1 in 1976, increasing and decreasing in no predictable pattern, leveling off at ~ 0.4 pg cm-2 yr-1 after 2000 before increasing rapidly to 1.31 pg cm-2 yr-1 in 2008. PFOS has an irregular flux beginning with 0.24 pg cm-2 yr-1 in 1982, never exceeding 0.29 pg cm-2 yr-1 by1995 before increasing to 1.23 pg cm-2 yr-1 in 2008. Although PFOA has the second-highest burden, its short record begins with 0.77 pg cm-2 yr-1in 1990, doubling to 1.69 pg cm-2 yr-1 by 1993, but not observed again until 2002 when it dropped by nearly 4 times (0.46 pg cm-2 yr-1). It nearly doubled by 2006, and increased by > 4x by 2008 (3.93 pg cm-2 yr-1). The dominance in burden by PFNA and PFOA is consistent with high concentrations of these PFCs observed in polar bear liver from Greenland.

EC02B-1
The deposition and fate of perfluorinated alkyl substances (PFAS) in the Norwegian Arctic snowpack
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Poly- and perfluorinated alkyl substances (PFAS) are man-made chemicals that are ubiquitous in the environment and are present in humans and wildlife. These chemicals occur in the Arctic through long-range transport processes with the perfluorinated acids (PFAs) [e.g. carboxylates (PFCAs) and sulfonates (PFSAs)], present in sentinel

organisms such as the Polar bear (*Ursus maritimus*) and the ringed seal (*Phoca hispida*). PFAs are present in arctic media through a number of processes, including transport with ocean currents, oxidative degradation of 'neutral' precursors (e.g. fluorotelomer alcohols) in the atmosphere and possibly transport with airborne particles and aerosols. A combination of these processes results in their deposition with snowfall, although only a few studies to date have examined accumulation of these chemicals in the Arctic snowpack Questions arise regarding the sources of these chemicals in atmospheric deposition in remote locations and the role of snow in delivering PFAs to catchment systems during periods of melt. In this study, a detailed examination of PFAS was undertaken in the seasonal snowpack at remote terrestrial sites in Northern Norway. The purpose was to investigate, in some detail, their accumulation in different snow layers and to relate their profile and concentrations to physical and chemical characteristics of the separate snow layers as well as their accumulation history. PFAS measurements in air (gas phase and particle-bound) and snow (dissolved and particulate matter) were conducted. Air mass trajectory analysis was used to examine air mass origins for specific air samples and snowfall events. In the snowpack, the vertical evolution of snow density, snow layer hardness, crystal morphology (macrophotography) was examined alongside temperature, pH, conductivity, organic matter content, major anions and cations, as well as particle characteristics, to help understand sources of PFAS to fresh snowfall and their fate during snow ageing. Initial results show large variations of pH and particle matter content between snow layers, with pH measurements as low as pH 4.68 in some layers, indicating the potential for a fraction of the PFAs to exist in their neutral acid form and hence be 'remobilised' during thaw periods.

EC02B-2

Secondary emissions of legacy pollutants and their pathways to the Arctic under a changing climate

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After the phase-out of a persistent chemical it can be expected to endure in the environment for some time, up to several decades. How long and where it will persist depends on its emission history and transport, partitioning between environmental compartments, and degradation. With respect to the accumulation of legacy pollutants the Arctic is a particularly interesting region. It is also in the Arctic, where future climate change is expected to affect most the presence of persistent pollutants.
The mechanisms leading to a particular global distribution of the contaminant are addressed by multimedia chemical fate models. These tools provide not only quantitative estimates of the global distribution of a chemical, but also an understanding of the pathways involved, and allow forecasts under different emission and climatic scenarios. In this work we model concentrations of some representative persistent organic pollutants in the Arctic after their phase out. In particular we indicate the timescales that can be expected for those chemicals to persist in the Arctic, and analyze the relationship between Arctic receptor sites and global secondary sources. Finally, we investigate the effect that the projected climate change may have on levels in the Arctic and the global distribution of secondary sources.
We show that beta-HCH and PCB153 will persist in the Arctic Environment for several decades after cessation of primary emissions with apparent half-lives of about 8 and 28 years, respectively. These results are only slightly sensitive to forecasted climate change. We further demonstrate that the location of secondary sources important for the Arctic is strongly dependent on the chemical properties of each pollutant.

EC02B-3

Spatial and temporal trends of persistent organic pollutants and mercury in ringed seals from the Canadian Arctic

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The goal of this ongoing study is to determine temporal trends of legacy POPs, new/emerging POPs, as well as mercury (Hg) in Canadian Arctic ringed seals using annual collections at Arviat, Resolute, and Sachs Harbour as well as less frequently at nearby communities. The study builds on results for legacy POPs and Hg going back to the 1980s, and earlier in some cases. Results for PBDEs, HBCD, perfluorinated chemicals (PFCs), and endosulfan, along with δ13C and δ15N data, have been added to samples collected since 2001 and on selected archived samples from the 1970s and 1990s. Sample collections (10 to 25 adult ringed seals; blubber, liver, muscle, tooth/lower jaw for aging) are carried out by local hunters each year (June-October). For neutral POPs, only blubber of females and juveniles are analysed to limit the influence of age. For Hg, muscle and liver samples are analysed, while PFCs are also determined in liver. Highest Hg concentrations in seal muscle and liver (age ≥ 5 yrs) were found in western and Central Arctic. No significant increase or decline of Hg in seal muscle was found over a 7 to 9 year period at Arviat, Resolute and Sachs Harbour. δ13C and δ15N in seal muscle varied only over a narrow range (±0.5 ") indicating little change in diet over the same period. Hg concentrations in liver were more variable over the same period. This was not related to diet or to age but could reflect more recent diets of individual animals compared to measurements of muscle N. To assess time trends of legacy and new POPs we combined data for nearby communities. Overall, there are declining trends in all regions with the relative magnitude of ΣDDT >αHCH >Σ10PCB >ΣCHL. Largest declines of all legacy POPs were in Hudson Bay possibly reflecting proximity to source regions in North America. PBDEs, PFOS, and PFCAs show increasing concentrations in the 1990s to early 2000s and then recent declines. Endosulfan and HBCD were present at low concentrations in seal blubber (0.01-2.0 ng/g) and appear to be increasing in concentration over the period 2005-2010. Temporal trends of legacy POPs in the Canadian arctic generally show declining trends. However trends for new POPs differ from those in Greenland particularly for PFCs. Continued annual sampling is improving the statistical power of the study and enabling testing of factors influencing trends of POPs and Hg including climate, diet and changes in global emissions.

EC02B-4

An observation-based mass balance assessment of PCBs in the Arctic Ocean

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The predictability of future distribution and exposure of PCBs in the Arctic hinges centrally on the quality of our understanding of the present-day distribution and processing of PCBs in the Arctic system. Real observations of PCBs in abiotic compartments in the Arctic Ocean are scarce, increasing the importance of model simulations for understanding the fate of PCBs in this extreme but sensitive environment. The objectives of the current study were to a) present an observation-based inventory of PCBs in the Arctic Ocean (AO), b) to relate observations to total global emissions and modeled simulations of transport to the Arctic, offering an assessment of how well we understand transport and fate of PCBs in the Arctic today, and c) to present a mass balance for the entire AO, identifying the major inventories, import and export fluxes of PCBs.
Water samples were collected covering all the water masses of the central AO (CAO) and surface water from the polar mixed layer in all seven shelf seas. Observed concentrations in combination with annual global emissions for the years 1930-2000 were used to estimate concentrations back to 1930. The total ∑PCB7 inventory for all Arctic Ocean water masses and ice was 64 t. The largest reservoir was the Arctic deep water, containing 77% of the total inventory. All water masses were dominated by tri- and tetra-chlorinated PCBs (40-70%). The Arctic inventory of PCB 52 corresponds to only 0,5% of the global total emission, contrasting to numbers of more than 5 % suggested by model simulations. Hence, a redistribution of PCBs to the atmosphere from the AO should not have a major impact on human and environmental exposure globally.
River discharge was a major input pathway (26-56%) to the AO during 1930-2000. The inventory for PCB 52 (including sediments) of 31 t fits well to the mass balance of 34 t imported and 16 t exported to the Arctic Ocean during 1930-2000, considering the uncertainties in these calculations. The average residence time of the Atlantic water in the CAO of approximately 25 years allowed this water to accumulate PCBs. Hence, water from the Atlantic layer exported from the AO contains higher concentrations of PCBs than the water that entered the AO, potentially offering a partial explanation for the discrepancy between model simulations and observation-based calculations presented here.

EC02B-5

Exposure of persistent organic pollutants in avian top predators in a changing northern climate

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Top predators in the Arctic ecosystem are the final destination of bioaccumulative POPs and mercury, and in such organisms the negative impacts are most likely to occur. However, little is known about how climate change will affect the accumulation of POPs in top predators. Changes in POPs in arctic top predators may occur by two pathways: 1) directly through increased POP transport or 2) indirectly through changes of POP uptake in the food chain and changes in diets of top predators. The importance of these pathways is little understood, and the aim of this presentation is to discuss how changing climate and feeding ecology may affect the uptake of POPs by using different examples on avian top predators in the arctic and upper temperate regions. Relationships are shown to factors both related to POP transport and to indirect mechanisms such as local climate and feeding conditions.

EC02B-6

Persistent organic pollutants (POPs) in Svalbard water food webs: is the Arctic far enough?

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Persistent organic pollutants (POPs) are semi volatile and have high environmental half-lives. These characteristics produce a long-range atmospheric transport and global planetary distribution, reaching locations far away from the pollution source. The overall process results in an enrichment of concentrations of some POPs in polar ecosystems, which became a global sink due to their environmental conditions. As a consequence of their biomagnification properties, high trophic food web organisms

provide a great opportunity for the analysis of background concentrations of POPs accumulated in Arctic biota.
Two water ecosystems from Svalbard archipelago (Arctic) were selected encompassing freshwater and marine fish as a targets (summer 2010). 18 Arctic char (Salvelinus alpinus) and two local marine fish species (17 Cod (Gadus morhua) and 9 Haddock (Melanogrammus aeglefinus)) were sampled.
Traditional persistent organic pollutants (PeCB, HCB, HCHs, DDTs, PCBs) were analysed in all samples and recently introduced compounds such as the polybrominated diphenyl ethers (PBDEs) have also been assessed. Arctic char showed higher heterogeneity in the abundance of groups of POPs (83-95% for PeCB, HCB, HCHs and DDTs and 50% for PCBs) while in marine species these compound groups were found in 100% of the samples. Mean concentrations of these first 5 groups ranged between 0.4 and 17.8 ng/g. Higher values were found for HCHs in all species. This could be explained by the high solubility and volatibility of these compounds in relation to other compounds.
For the PBDEs, at least one congener in each sample was quantified in 39% of Arctic chars and in 35% of marine samples. Concentrations of total PBDEs ranged between 0.004-0.306 ng/g; 0.010-0.177 ng/g and 0.005-0.538 ng/g in Arctic char, Cod and Haddock, respectively. The most abundant congeners were PBDE-47, PBDE-99 and PBDE-209.
The results from our work confirm a background level of organochlorine compounds in Arctic regions. Further studies should also include other emerging organic compounds of concern in freshwater and marine biota.

EC04 - Novel approaches to addressing metal and metal nanomaterial bioavailability in soils

EC04-1
Validation and first deployment of the DGT technique in artificial human gastrointestinal fluids after ingestion of metal-containing soil particles
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The accumulation of metals in soils due to human activities constitutes a potential health risk if directly ingested, especially by children via hand-to-mouth behaviour. Contaminants can be partially or totally released from soil by ingestion, depending on their speciation under gastrointestinal conditions. In vitro tests provide estimates of bioaccessibility, defined as the proportion of contaminant that is dissolved in the artificial gastrointestinal fluids and is potentially available for absorption. The flux toward the intestinal membrane corresponds to both the free metal ion and labile metal species. In contrast, inert species cannot dissociate and thus do not contribute to transport across the intestinal membrane. Estimation of the labile fraction can be assessed with the Diffusive Gradient in Thin films (DGT) technique. Whereas the effectiveness of DGT has been demonstrated for various metals (Cd, Zn, Cu, Ni, Pb) in different exposure media (natural waters, soils and sediments), no data is available in artificial human gastrointestinal fluids. The objectives of this study were firstly to validate the performance of the DGT technique for Cd, Pb and Zn in controlled digestive solutions for different times of exposure and different metal concentrations, and secondly to use the technique in the gastrointestinal solutions obtained after carrying out the in vitro Unified Barge Method (UBM) test on highly contaminated soils. The results demonstrated the suitability of the DGT technique for Cd, Pb and Zn measurements in the gastrointestinal media with linear response and stable accumulation up to 6 hours at 25°C and then at 37°C. Combining the in vitro test with the DGT technique provided an approach to the labile metal species available for transport across the intestinal epithelium. Thus, the gastrointestinal absorption of ingested metals ranged from 8 to 30% for Cd, 0.6 to 11% for Pb, and 0.8 to 7% for Zn and was influenced by metal speciation. In this original approach, the DGT technique was found to be simple and reliable in the investigation of labile metal species in digestive fluids. Extrapolation to the in vivo situation should be undertaken very cautiously and requires further investigation.

EC04-2
Comparison of the determination of free Zn(II) concentration in soils using AGNES and DMT
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Key paradigms in environmental sciences, such as the Free Ion Activity Model (FIAM) or the Biotic Ligand Model (BLM), attribute a central role to the free metal ion concentration (rather than the total metal concentration) in the toxic or nutritional effects of a particular element. Consequently, there is a significant interest for analytical techniques that can act as selective probes for the free Zn(II) concentration in a large variety of environmental systems, and, specifically, in soils. The measurement of free Zn(II) concentration is particularly challenging, because there is no commercial Ion Selective Electrode for this element. Only a very few techniques have a direct access to the free metal ion concentration, while many other popular techniques (such as DGT, Diffusion Gradients in Thin Films) measure operationally defined fractions (e.g. a certain labile fraction). AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) and DMT (Donnan Membrane Technique) provide robust and direct measurements of the free metal ion concentrations. In the literature, AGNES has been applied for the determination of free Zn in seawater, freshwater, humic acid solutions, ZnO nanoparticles dispersions, etc. DMT has been applied to soils, natural waters, etc.
In this work, the application of both techniques to the same synthetic and natural systems allows a cross-validation. AGNES and DMT are validated in synthetic solutions of Zn+NTA, yielding results in agreement with each other and with the theoretical code VMINTEQ. A further validation came from analysis of Rhine river water. A critical comparison of the characteristics of both techniques can be performed in terms of time of analysis, limit of detection, required instrumentation, etc.
This work is the first application of AGNES to different type of soils extracts. The analyses of free Zn in 4 soil extracts (rivier clay, cover sand, loam and reclaimed peat; all from the Netherlands) also gave similar concentrations with both techniques, and consistent with ECOSAT theoretical predictions. The impact of the small pH drift in DMT donor solutions is negligible. The percentages of free Zn (with respect to the total zinc concentration) range between 40% and 80%. This indicates that, in the four considered soils, Zn is much loosely bound than Cu, which, in principle, should imply a larger bioavailability of Zn (II) to plants and animals.

EC04-3
Using radioactive and stable metal isotopes to study metal and metalloid availability and ecotoxicity in soils
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Methods to study the speciation of metals in the environment has progressed rapidly over the last few decades, with the principal advances being in aqueous phase methodologies. In soils (and sediments), speciation of metals in the solid phase is problematic, with classical sequential fractionation schemes being operationally defined and having several drawbacks. Synchrotron x-ray spectroscopy has opened up new avenues to examine solid phase speciation of metals in soils, but suffers from the drawback that it cannot quantitate metal availability and behaviour, which must be inferred from knowledge of solid-phase forms identified. Isotopic methods can be used to either trace metal/metalloids in particular forms added to soils, or isotopic dilution can be used to examine the fate and behaviour of materials that cannot easily be isotopically labelled (e.g. manufactured materials or wastes).
Isotopic methods provide an extremely valuable tool to probe the fate, behaviour and biological availability of metals and metalloids in soils. The information provided by isotopic methods has already been used in regulatory frameworks for metals risk assessment [and will continue to be a vital tool in probing metal and metalloid behaviour in soils. Looking to the future, new methods examining the differences in natural abundance of metal/metalloid isotope abundance will open up our understanding of metal/metalloid availability in soils

EC04-4
Stable isotopes for micronutrient metal bioavailability to earthworms
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In urban environments two of the most common metal contaminants are copper and zinc. In addition to being contaminants, these metals are regulated micronutrients in most organisms. Both have stable isotopes (^{65}Cu and ^{68}Zn) that are readily quantified and differentiated from the more abundant isotopes (^{63}Cu and ^{66}Zn) by modern instrumental techniques (ICP-MS). There are two parts to our previous studies, first we hypothesized that by modifying the metal isotopic ratio in a soil, we could elevate the ratio of ^{65}Cu/^{63}Cu or ^{68}Zn/^{66}Zn in an earthworm (either Eisenia fetida or Lumbricus terrestris) living in that soil in a short period of time. Second, after increasing the isotopic ratio, the change in that ratio after placing the worm into a soil with a normal isotopic ratio could be used as a metric of bioavailability. The change in ratio would be a result of either an exchange of stable isotope for the more abundant isotope or the dilution of the ratio due to accumulation of the abundant isotope; in either case the ratio would decrease proportional to the metal available from the soil to the organism. Our research using stable isotopes has shown usefulness in determining micronutrient metal bioavailability in soil systems. With both Cu and Zn, isotopic ratios were increased 6-8 fold over background ratios by exposing worms to labeled soils. When placed back into soils with natural isotopic abundances ratios returned to near background levels. Understanding differences in how organisms store and utilize these metals is important if these methods are to be further developed. Zinc isotopes show promise due to its longer term storage in the anterior organ tissues of L. terrestris, while the extremely fast turnover rate of copper in E. fetida suggests that stable isotopes may not be as useful as first hypothesized.

EC04-5
Weathering of silver nanoparticles could increase their bioavailability
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The major challenge in tracing engineered nanoparticles (ENPs) in complex media, such as soils, is to detect their presence, transfer to organisms and their interactions with

the surrounding environment. One possibility to overcome this issue is to use neutron activation where ENPs are subjected to a strong neutron flux that induces a more or less transient radioactive property in the ENPs without changing other chemical or physical properties that influence their behaviour during experimentation. Radioactive ENPs are subsequently detected and quantified by counting of gamma rays emitted by the isotopes that are formed.
To date, an important amount of toxicity data on ENPs is available, but data on exposure are still needed to be able to conduct risk assessment. In a first experiment, we followed the uptake and excretion of Ag from either AgNPs or AgNO3 in the earthworm Eisenia fetida. In a second experiment, we studied the partitioning of Ag from either AgNPs or AgNO3 in two natural soils of contrasting organic matter content, and over time.
At the end of the exposure period (day 28), earthworms had body concentrations corresponding to 5.1 ± 0.5 % and 11.0 ± 0.3 % of the concentration in the food for AgNPs and Ag ions, respectively. These values decreased by 80 % and 93 % within 48h depuration in clean soil, for AgNO3 and Ag NPs, respectively. After two months depuration, 97 % and 99 % of the accumulated Ag from Ag ions and AgNPs, respectively, were excreted. Bioaccessible Ag was defined as the sum of Ag extracted by water and ammonium acetate. A rapid reduction of Ag ions occured when they get in contact with soil organic matter, turning Ag ions into NPs and colloids. Soil properties had a limited impact on Ag speciation. Interestingly, the bioaccessible fraction increased over time in case of AgNPs, contrary to what happened with AgNO3. We showed that the studied AgNPs were more bioaccessible than Ag ions over time, since they could act as a constant source of relatively stable and bioaccessible Ag. This increase in bioaccessible Ag in soil spiked with AgNPs, being between 8-9 times greater than the bioaccessible fraction of AgNO3 after 70 days contact time, calls for caution in ENP risk assessment, and the low bioavailability of AgNPs to earthworms should be re-evaluated in light of the increase of silver bioaccessibility as particles weather in soil.

EC04-6
Evaluating the impact of soil ageing on the toxicity of Ag nanoparticles to Eisenia fetida
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The increased production and use of engineered nanoparticles in a variety applications increases the potential for exposure to these particles in the environment. Currently, very little is known about how metal nanoparticles behave once released into the environment and even less is know about the effect of these particles on terrestrial organisms. The purpose of this investigation was to characterize the difference in toxicity and the impact of ageing between silver nanoparticles and silver nitrate in OECD soil on the earthworm Eisenia fetida. The potential to modify the toxicity of a silver nanoparticle contaminated soil over time is an important temporal consideration when conducting a terrestrial risk assessment.
A set of Earthworms were exposed to the AgNO3 and AgNP spiked soils 24 hours after initial preparation and then a second set of earthworms were exposed to the soils aged 28 days. Both exposures lasted 14 days with endpoints of mortality and body burden. OECD soil treatments were prepared with dissolved AgNO3 or suspensions of 30-50 nm Ag nanoparticles (NP). Soil treatements were then split and one batch was allowed to age for 28 days in an incubator and kept at constant moisture. The aged soils were sampled every 7 days and subjected to a sequential extraction procedure to evaluate changes in Ag speciation.
Results from the first exposure showed high mortality for worms exposed to silver nitrate and almost no mortality for those individuals exposed to Ag nanoparticles. The exposure to soils aged 28 days showed a decrease in mortality in individuals exposed to the AgNO3 treatments and an increase in mortality in those exposed to the aged AgNP soils. The Ag body burden for both AgNP exposures were indistinguishable while there was a decrease in the Ag body burden for earthworms in the AgNO3 exposures aged 28 days as compared to freshly amended soils. There were changes is the speciation of Ag over the 28 day ageing period in both soil treatments that point toward an increase in the potentially bioavailable pool of metal in AgNP amended soils as compared to the AgNO3 amended soils. Based on these results, the potential exists that morphological changes to the AgNPs in the soil matrix occurred during ageing and that these changes in conjunction with more bioavailable Ag made these treatments more toxic to exposed individuals.

EC05 - Occurrence, fate and impact of atmospheric pollutants on environmental and human health

EC05A-1
Application of flow-through air samplers and passive air samplers to assess the vertical variability of semi-volatile organic contaminants in the atmosphere of Hawaii
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Many semi-volatile organic chemicals (SVOCs) such as organochlorine pesticides and polybrominated diphenyl ethers (PBDEs) have been ubiquitously detected in the global atmosphere, even in remote regions where such chemicals have never been produced or used. While many studies have focused on SVOCs transported to remote polar and highland regions, few studies have investigated SVOCs undergoing long range transport to remote tropical islands far from emission sources. The global long range atmospheric transport of SVOCs occurs mainly in the free troposphere, where little interaction with the earth surface boundary layers occurs. Investigating the occurrence of SVOCs in the free troposphere is important to understand their long range atmospheric transport. In this study, air from the free troposphere and from the marine boundary layer of the Big Island of Hawaii were sampled at Mauna Loa (19°43'53'N, 155°2'52'W, 3398 m above sea level) and at the east coast of the island (19°32'9'N, 155°34'31'W, sea level, 50 m horizontal distance from the ocean) using flow-through air samplers (FASs). FASs were operated simultaneously at the two sites from April to September, 2011. Polyurethane foam (PUF) plugs used as the sampling medium were replaced every month. Three PUF plugs were installed in each FAS and analyzed separately to assess and correct for break-through. Each month, 3100-7700 m^3 and 300-800 m^3 of air was sampled at the Mauna Loa site and at the coastal site, respectively. Among the PBDEs analyzed in the samples using an Agilent 7000A triple quadrupole GC/MS/MS system, only BDE-47 and -99 were detected frequently over the five months. The concentrations in the marine boundary layer (4.4-24 pg/m^3 BDE-47 and 2.4-88 pg/m^3 BDE-99) were higher than in the free troposphere (0.1-0.4 pg/m^3 BDE-47 and 0.1-0.3 pg/m^3 DE-99) PBDE concentrations measured at the mountain site were lower than what has been reported for other global background sites in the Arctic and on the Tibetan Plateau, suggesting that Mauna Loamay truly represent global background levels for SVOCs. Between the two FAS sampling sites, within a horizontal distance of 50 km, the elevation changes from 0 m to 4000 m above sea levels. Along this large gradient, we also deployed XAD-based passive air samplers to assess the vertical variability of SVOCs.

EC05A-2
Atmospheric deposition of polychlorinated naphthalenes in Dongjiang River Basin of Guangdong province, South China
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Polychlorinated naphthalenes (PCNs) are a group of chemicals consisting of naphthalene substituted with 1-8 chlorine atoms, which were historically used for their thermal stability in dielectric fluids and insulators. PCNs are no longer commercially produced ever since 1977, but they are still routinely observed in the environment, and have been identified as persistent, toxic, and bioaccumulative substances. Atmospheric deposition of PCNs was investigated at 10 sites in Dongjiang River basin (The eastern Pearl River Delta) of Guangdong Province South China, during the winter and summer of 2010. The average daily deposition flux of total PCNs was 828 pg/(m^2 d), and the corresponding TEQ was 0.14 pg/(m^2 d). Based on these values, the presumed average annual deposition of total PCNs was 8.5 kg for Dongjiang River basin within Guangdong province, and the corresponding TEQ was 1.3 g. Tri-CNs dominated the deposition fluxes in all samples and contributed to more than 50% of total PCNs. In addition, high contents of more chlorinated PCNs (penta-CNs to octa-CN) implying the source areas were discovered in Guangzhou and Dongguan instead of Huizhou. Spatially, the average daily deposition fluxes of PCNs in Guangzhou city and Dongguan city were much higher than those in less developed Huizhou city. Seasonally, the fluxes were generally higher in summer than in winter, while the corresponding TEQ fluxes were the reverse. The results indicated the PCN emissions in the Dongjiang River basin could be ascribed to both the combustions and other sources, while to contemporary PCN the combustion sources was becoming important. Few studies have focused on the atmospheric deposition of PCNs, so the results can be useful for evaluating the potential effects of PCNs to the Dongjiang River.

EC05A-3
Environmental monitoring networks are important tools to assess chemical regulations
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A recent expert meeting organized by the RECETOX Centre in Brno, Czech Republic, has identified gaps, challenges, and research needs associated with the global assessment of hazardous chemicals. One of the important areas of concern recognized by this workshop was a need in developing a range of techniques addressing an improved assessment of spatial and temporal trends of the chemicals of global concern. Establishment of carefully designed environmental monitoring networks can be an important tool in the assessment of trends of toxic compounds in the environment and development of policy regulations on production and use of these chemicals, as well as testing the effectiveness of already existing regulations. The goal of this paper is to present an example of a North American environmental monitoring network, emphasizing methodological and technical details, as well as the results of these monitoring efforts. The Integrated Atmospheric Deposition Network (IADN) is a joint monitoring and research program between the United States Environmental Protection Agency and Environment Canada. IADN was begun in 1990 through mandates of the Clean Air Act and the Great Lakes Water Quality Agreement to monitor the atmospheric deposition of persistent organic pollutants into the Great Lakes. IADN flame retardant data for 2005-2009 time period will be presented here as an example of how environmental monitoring data can be used to assess temporal and spatial trends of persistent chemicals. Overall, Chicago and Cleveland have the highest concentrations of PBDEs, BTBPE, and DBDPE in all three phases, suggesting a strong urban atmospheric source of these pollutants. The two remote sites, Sleeping Bear Dunes and Eagle Harbor, have the lowest concentrations of these contaminants. The remote site at Eagle Harbor had particularly high levels of PBEB in all three phases, and the rural site at Sturgeon Point had the highest HBB concentrations in the vapor phase. To investigate temporal trends

of these compounds in the period of 2005-2009, a multiple linear regression was applied to the concentrations of these chemicals in all three phases combined together. PBDE concentrations showed decreasing trends over time, with halving times of ~6 years. The concentrations of HBB and BTBPE are decreasing with halving times of ~10 years. PBEB and DBDPE concentrations did not show any change between 2005 and 2009, suggesting continuing source for these compounds.

EC05A-4

Assessment of persistent organic pollutant in the atmosphere of Latin America

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The widespread use and distribution of pesticides, industrial and urban chemicals and the consequent release into the environment, is of great worldwide relevance. Atmospheric transport is responsible for pollutant dispersal over long distances. As part of an atmospheric regional network, a monitoring program involving the use of pine needles, epiphytes and passive samplers is conducted. Pine needles (Pinus sp.) can accumulate hydrophobic compounds such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyls ethers (PBDEs), from air. Moreover, they integrate contaminants loads over a long time. All these compounds, included in the Stockholm Convention, are of concern due to their distribution, global transport and toxicity. Pine needles were used to evaluate latitudinal and longitudinal transport of contaminants in central and Patagonian regions from Argentina, with a 20 stations network. The epiphyte Tillandsia bergerii was used to evaluate local sources at small scale, setting 7 transplanting points including urban, periurban and rural land uses. Passive samplers (XAD-2) constitute the aim of our Latin American Atmospheric Passive Sampling Network (LAPAN) where 46 sites are actually covered while 80 sites are at least our endeavor to reach. The network involves regions of Antarctica and 12 countries including urban, agricultural and industrial areas. Analyses are performed by GC-ECD and GC-MS. Pine needles analyses revealed that among OCPs, the currently used endosulfans, are the main pesticides found, particularly associated with agricultural areas, however DDE, the DDT metabolite, is also found in all sites independently of particular sources, as a consequence of their intensive past use in the region. Regarding PCBs, a predominance of #110, 118, 153, 138 is found, related with punctual sources. Considering the latitudinal gradient, until the southeast region of Argentina, an increasing in DDT, PCBs and PBDEs (BDE-28 and 47) is observed inferring an atmospheric transport of these compounds. PBDEs and PCBs levels in T. bergerii showed a concentric distribution around the urban settlement with a clear hot spot near a waste disposition site, that is accomplished by the presence of PBDEs 28, 47, 99 and 100 and PCBs 153, 138, 110 and 118. In all industrial or urban sites the relation PBDEs/PCBs >1 reflect the general trend of diminishing PCBs levels and increasing the emergent PBDEs

EC05A-5

Occurrence of currently use pesticides and selected degradation products in agricultural regions of Western Canada

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Initial studies on occurrence of currently used pestides in agricultural regions of Western Canada focused on the Lower Fraser Valley where berries and fruits are the dominant crops, and the Canadian prairies where grains and oil seed crops dominate. These two agricultural regions were selected due to their differences in crop types, climate, and expected usage of pesticides. Western Canadian agricultural regions have the highest historical usage of pesticides in Canada. In the initial studies we focused on key pesticides that we suspected to be of concern and was prior to the availablility of a 2003 usage inventory. These pesticides had little information about their atmospheric occurrence. In the Lower Fraser Valley (LFV) we examined trihalomethyl thiofungicides and were able to detect captan and folpet, while in the prairies triallate, trifluralin, and ethalfluralin were determinined to be key herbicides that were still dominant in the atmosphere. From these studies the gas/particle partitioning of captan and folpet was also examined. Due to its lower vapour pressure, captan was found to be present in both the particle and gas phase, while folpet was only detected in the gas phase. Seasonal atmospheric concentrations in the LFV were dominanted by the particle phase concentrations of captan and showed the importance of examining atmospheric transport processes on particles. In the prairies we also provided the first detection of azole fungicides in the atmosphere in the gas phase and related these to precipitation during a wetter than normal year (2010). In addition we also examined the differences between the two distinct agricultural regions, and provided the first detection and seasonal trends of organophosporus oxon degradation products along with thier active OP ingredient in North America. Ratio of degradation product/active ingredient were also used to identify the age of the source and to provide insight into future studies on the relative importance of local, regional, and long-range atmospheric transport sources. In the next phase of the research we expanded the number of sampling locations in each of these agricultural regions starting in 2011 in the LFV and to an adjacent agricultural region, Okanagan Valley, which is more heavily dominated by orchards and vineyards. A summary of the currently used pesticides that are part of this 5-year study and some preliminary results will be presented.

EC05A-6

Semi-volatile organic pollutants and trace metals associated with Saharan dust air masses: estimated inhalation exposures at source and downwind sites

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Saharan dust air masses transport eroded mineral dust, chemical contaminants, and microorganisms in the atmosphere thousands of kilometers from the Sahara/Sahel of Africa to the Americas, Europe and Asia. Global ocean-atmosphere interactions, regional meteorology, surface material composition, and human activities are the primary factors driving the composition and quantities of transported dust particles and associated contaminants. Semi-volatile organic compounds (SOCs) such as banned and current use pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) have been detected in dust air masses over source (Mali) and downwind locations (Cape Verde, Trinidad and Tobago, and U.S. Virgin Islands). All are known to persist in the environment, bioaccumulate and be hazardous to humans and other organisms. Some banned pesticides such as DDT are currently used in the source region against agricultural pests (locust plagues) and disease vectors (e.g., malarial mosquitos). Primary sources of PAHs include small, garbage-and-biomass-burning low temperature fires and vehicle exhaust (primarily diesel and two-stroke gasoline engines). Concentration data from source and downwind sites during Saharan dust conditions were used to estimate inhalation exposure to total and individual (14) pesticides, total PAHs, total PCBs, and biologically active trace metal(loid)s. Inhalation exposures ranged from 17-1085 nanograms per day (ng/d) for detected SOCs in the source region and <1-6 ng/d at downwind sites during dust conditions. Bioaccessible metal(loid) exposure ranged from 0-2519 ng/d Fe, and < 1-11 ng/d As in the source region and 55-2006 ng/d Fe and < 1-4 ng/d As at Caribbean sites. Because biological exposure to metal(loid)s is dependent on species accessibility, preliminary investigations of bioaccessibility of inhaled or ingested particle-associated trace metals were conducted using simulated lung and gastric fluids. Bioaccessibility varied (0-100%) among trace metals (and redox state), fluid (simulated lung or gastric), and by source-downwind location. Iron and potentially toxic elements (As, Cr, Cu, Mn) showed enhanced bioaccessibility in downwind (Caribbean) samples. Fe is of particular interest due to its ability to induce an inflammatory response. Saharan dust air mass samples contain multiple SOCs and biologically active trace metals, raising the question of possible synergistic negative effects on humans and other organisms.

EC05B-1

Re-visiting the modelling of soil-air partitioning, fugacities in soil, and soil-air exchange of persistent organic pollutants

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Soils are the main reservoir of persistent organic pollutants in the environment and air-soil exchange of POPs is a key process affecting the atmospheric occurrence of POPs and the extent of soil as a sink of pollutants. The direction of the air-soil exchange can be determined by comparing the POP fugacity in soil (fs) and the fugacity in ambient air (fa). If fs is higher than fa, then there is a net volatilization, while if fs is lower than fa then there is a net deposition. fs is usually estimated from the soil-air partition coefficient (KSA), which is often estimated from the octanol water partiiton coefficient. Lately, polyparameter liner free energy modles (pp-LFER) have also become popular for modelling the environmental partitioning of POPs. The recent development and aplication of a soil fugacity sampler allows for the first time to validate unequivocally these models. The objective of this work is to revisit the modelling of soil-air exchange by fitting this model to the most extensive database available for concurrent measurements of soil concentrations, fugacities in soil and fugacities in ambient air. The application and recommendation for modelling strategies will be provided dependent on the chemical properties, environmental parameters such as temperatures and soil characteristics. Implications for modelling soil-air exchange will also be given.

EC05B-2

Prospects for estimating atmospheric lifetimes of micropollutants using the Junge relationship

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The Junge variability-lifetime relationship relates the relative standard deviation of concentrations of trace gases to their atmospheric lifetime using empirical fitting parameters. Conceptually, the Junge relationship is a consequence of incomplete mixing of gases in the global atmosphere. At the limit, gases with very long lifetimes approach a homogenous distribution in the earth's atmosphere. More generally, variability in atmospheric concentrations of gases with shorter lifetimes measured at remote locations will be greater than variability of gases with longer lifetimes. Here, we present results of a pilot study that aims to take advantage of the ubiquitous presence of cyclic volatile methylsiloxanes (cVMS) in the global atmosphere to calibrate the Junge relationship and estimate atmospheric lifetimes for other pollutants. The cVMS have many advantages

as benchmark substances to calibrate the Junge relationship, namely; 1) They are high-production volume chemicals with high release rates and high concentrations in the atmosphere; 2) Their atmospheric lifetimes are well-understood based on a combination of laboratory and field studies that have been verified with mass balance modeling; and 3) they are emitted from urban areas, which coincides with source areas for many other atmospheric micropollutants of interest and opens the possibility of extrapolating Junge relationships for the cVMS to estimate atmsopheric lifetimes of other atmosphreic micropollutants. We have analyzed two datasets for evidence of variability consistent with the Junge relationship: Measurements at 12 sites of the Global Atmospheric Passive Sampling (GAPS) network, and measurements made at a rural site in southern Sweden, and found strong Junge relationships in both datasets. We view these relationships as proof-of-concept that the cVMS can be used to calibrate the Junge relationship and enable quantitative estimation of atmospheric lifetimes of organic substances if they are measured simultaneously and have similar source profiles and sink processes. Measurements of siloxanes and other selected pollutants in air at the full complement of over 50 GAPS sites is currently underway, and will provide a more extensive data set for testing the Junge relationships in future work. Particularly intriguing is the potential for applying the relationship to estimate atmospheric lifetimes of substances with significant sinks from dry and wet deposition.

EC05B-3
On the contribution of biomass burning to POPs in air in Africa
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Forest, savannah and agricultural debris fires in the tropics and subtropics are sources for wide spread pollution and release many organic substances into air and soil, including persistent organic pollutants, i.e. polychlorinated dibenzodioxins and -furanes (PCDD/Fs) and polycyclic aromatic hydrocarbons (PAHs). The significance of this source for the exposure of humans and the environment is unknown.
The global multicompartment chemistry-transport model MPI-MCTM [1] is used to predict atmospheric concentrations of selected PCDDs and PAHs. The model large-scale meteorology was constrained by nudging the atmospheric sub-model to re-analysis data. Global emissions of PAHs and PCDDs into air are based on recommended (PCDDs) and selected (PAHs) emission factors applied to fire distributions. Daily real-time fire data are based on satellite-observed fire radiative power measured from satellite (MODIS instrument) [2]. No other primary sources are considered. Model-predicted near-ground concentrations of PCDDs and PAHs are compared with observations during January-June 2008 at a number of stations across Africa [3]. Back-trajectory analyses suggest that some of these had been influenced by fire episodes in the region.
Continental half-year (Jan-June 2008) mean near-ground atmospheric concentrations are 0.0076, 0.51 and 3.25 fg m-3 of 2,3,7,8-TCDD, 1,2,3,4,6,7,8-HpCDD and OCDD, respectively. It is found that open fires can explain a major fraction of the air pollution by PCDDs in the background of west, central and southern Africa.
Highest concentrations of PCDD and PAH are predicted in 1-4 km altitude throughout most of the time, sometimes even higher and sometimes near the ground.
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EC05B-4
Tracing contaminants from urban sources to fate
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Cities can be major point sources of chemical emissions through air, surface water and waste water treatment plant discharges. Using Toronto as a case study, we found that the Central Business District held the greatest inventory of PBDEs and PCBs, had the highest air concentrations and the higehst emissions to air. These results speak to the importance of non-industrial sources. This source profile is poorly served by pollution emission regulations and hence point to the need for preventative approaches to minimize releases.

EC05B-5
Exposure to airborne pollutants - experience from Danish studies
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DK air pollutant (AP) levels are generally moderate due to windy climate and moderate emissions. Despite this, DK epidemiological studies points at severe negative adverse health effects: stroke, lung cancer, COPD, asthma in adults, wheeze in infants, asthma hospital admission in children, oxidative stress in blood DNA, vascular function in elderly, AP enhance effects of radon on childhood leukaemia, and most recently diabetes. These findings have been possible due to access to precise health data and advanced exposure assessment methods. Unique population and health registries in DK allow detailed health impact assessments to be carried out. Measurements from the DK AP monitoring programmes and AirGIS calculations on address level have been used as exposure proxies in a series of publications to evaluate various negative health outcomes. The later were based either on data from health registers or biomarker measurements. For assessing exposures, a GIS based modelling system, AirGIS (www.AirGIS.dk), has been developed. AirGIS is originally aimed for traffic air pollution, but is under steady improvement and development e.g. also to handle other pollutant emissions. The central part is Operational Street Pollution Model (OSPM), currently applied in >17 countries worldwide. Within the Danish research centre AIRPOLIFE (www.airpolife.dk), the AirGIS system was applied for exposure assessment for a variety of DK cohorts including the diet, cancer, & health cohort of 50,000 people. Wood smoke is the largest source of particles emissions in Denmark, and health effects have been studied in chamber experiments, but not yet in epidemiological studies. Wood smoke and emissions of aeroallergens (e.g. pollen, fungal spores, free allergens etc) from either agricultural activities or from vegetation are among the future aims for further development of AirGIS. Air pollution is believed to increase allergenic potential of airborne pollen and risk of new sensitisation. Traditionally, monitoring of aeroallergens is performed solely through sparse networks. Recent field studies have investigated exposure variability across an urban environment, and related chamber experiments have been applied to dose-response relationships for asthmatic people. Modelling strategies used in air pollution monitoring are currently being modified for aeroallergens, where the long-term goal is to incorporate aeroallergens within the AirGIS.

EC05B-6
Insight into the primary and secondary organic fraction of the organic aerosol in an urban area: Barcelona
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Monitoring and chemical analysis of atmospheric fine particulate matter (PM1) is important due to its health impact and influence on climate change (1,2). The air quality in the urban area of Barcelona in the Western Mediterranean Basin is assumed to be dominated by traffic related emissions and characterized by high levels of particulate matter and reactive chemical species due to emissions, the weak synoptic conditions and high solar radiation (3,4). Ambient air filter samples were collected during intensive sampling campaigns during 2009 and 2010 (DAURE and SAPUSS) on urban background (UB) and road sites (RS) and a rural background at 780 m (RB). The samples were analyzed for organic tracer compounds, e.g. polycyclic aromatic hydrocarbons, hopanes, alkanes, hydrosugars and nicotine, as well as secondary organic aerosols tracer compounds, e.g. dicarboxylic acids, for source characterization and identification of the organic patterns of primary and secondary aerosols. The obtained are compared with "on-line"data, such as those generated with Aerosol-Mass-Spectrometer. The results are discussed in terms of their relation to emission sources and influence of meteorological conditions in order to get an insight on the source contributions to the complex organic aerosol [5].
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EC06 - Sorption and bioavailability in sustainable remediation of organic chemicals

EC06A-1
The role of bioavailability in risk reduction of contaminated sites

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Most regulations and regulatory accepted assessment procedures on soil and sediment contamination are still based on total concentrations. When removal of the contaminant is an expensive exercise and/or there are doubts on the risks posed by the contaminants this often leads to a "wait and see"attitude. Site investigations are often repeated, but no actions are taken to reduce the risks of the contaminants. From a risk-based point of view, contaminations are only a risk if they are or may become (bio)available. This widens the range of management options of contaminated sites and can facilitate more tailor-made solutions for individual sites. In a risk based approach stimulation of biodegradation and/or immobilization and isolation of the contaminant may play a role. In particular bioavailability can be the underlying basis for the description of risks and for determining a solution and can be used to break the infinite circle of new site investigations.
Bioavailability should be more than a concept and including bioavailability in site management asks for methods to measure the bioavailable fraction. Such methods should have an understandable physical base (ISO 17402) and are fortunately available. Using the Tenax method the available and therefor degradable fraction can be measured. Using the different available fractions it is possible to predict the rate of degradation of PAHs and mineral oil and depending on this rate a management plan can be developed. Background and applications will be shown on remediation of heavily and also slightly contaminated soils and sediments.
If contaminants are not biodegradable, the bioavailability can be reduced by immobilization, for instance by adding black carbon or by preventing leaching and physical contact by isolation. Examples will be presented on the management of remote pesticide contaminated areas in Africa using charcoal for immobilization. Natural dune formation and/or stimulation of vegetation to evaporate water thereby preventing leaching have been used for isolation.

EC06A-2
Effect of activated carbon amendments on microbiological communities in PAH contaminated urban soil
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The addition of activated carbon (AC) to contaminated soils is currently being investigated as a cost-effective remediation technique, and an important consideration is the long-term effect of the AC amendments on microbial communities involved in the biodegradation of the organic pollutants. In this work, urban soils impacted by 23±15 mg per kg polycyclic aromatic hydrocarbons were sampled from a remediation field trial, and the long-term effect of 2.0% powder (PAC) or granular (GAC) activated carbon amendments on the microbial community structure and functioning was studied, by using molecular techniques. Denaturing gradient gel electrophoresis (DGGE) analysis showed a statistically significant shift in the predominant microbial community in the soils over time, whereas the effect of PAC or GAC amendments was not statistically significant in an ANOSIM comparison. After three years, the total microbial cell count and soil respiration rates were highest for the GAC amended soils, but cell numbers and respiration rates agreed within a factor three. The sequencing of the predominant DGGE bands, which had similar relative intensity in all soils, revealed the presence of taxa with closest affiliations to known PAH degraders (ie. Rhodococcus jostii RHA-1), or taxa known to harbour PAH degraders (ie. Rhodococcus erythropolis). The potential of the microbial community to degrade PAHs was evaluated by quantifying specific dioxygenase genes, using real-time polymerase chain reaction (PCR) assays. Similar gene copy numbers were measured in unamended, PAC and GAC amended soils. Polyethylene (PE) passive samples batch studies showed a reduction of the PAHs availability with biodegradation when comparing live and sterile soils. The strongest effect of biodegradation on PAH availability was found in unamended soil, with 75% difference between sterile and live soil slurries, while the lowest PAH availability was measured in PAC amended, live soil. The combination of the chemical and microbial studies suggested that microorganisms with the ability to degrade PAHs persist long-term in soils, regardless of the presence of activated carbons amendments which reduce the PAH availability, presumably because they utilized other soil organic matter as their main carbon source.

EC06A-3
Effects of carbon amendments on ecological responses and PCB bioaccumulation in Lumbriculus variegatus
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During the recent years synthetic black carbon products (mainly activated carbons AC, but also biochars), have been under an intense study to be used as a potential stabilization method for contaminated sediments. Results from various studies both in laboratory and pilot scale field studies have supported the high efficiency of AC amendments in sorption, and consequential reduction in bioavailability of HOCs. However, recent studies also indicate that AC amendments may inflict adverse ecological effects on aquatic organisms, e.g. sediment avoidance, inhibition in growth and alteration in sediment ingestion. The direct biological effect of AC on organisms may have an influence for both laboratory-scale bioaccumulation and toxicity testing and field-scale ecological impact assessment. The aim of this study was to test the responses of carbon amendments for remedial purposes in oligochaete Lumbriculus variegatus, a widely used test organism in sediment ecotoxicology. The effects of carbon amendments were tested in PCB contaminated sediments, which do not have acute toxic effect on the test organisms. The measured parameters included PCB bioaccumulation, feeding activity, growth and reproduction.
The AC was more efficient in reducing the bioavailability of PCBs than the tested biochar and the reductions were sediment specific. The results showed a clear AC dose related response in all of the studied ecological parameters. The ecological responses were also sediment dependent. The AC also reduced the biomass of the worms compared to the control; moreover in two sediments out of three the AC addition in the sediment ultimately led the worms to lose weight during the experiment period, whereas in control sediments the worms were gaining weight. The effects of biochar on the biological responses were smaller than that of the AC. The site-specific evaluation is particularly important when remediation measures are designed. Negative effects of carbon amendments to the organisms, such as change in behavior, reduced growth and reduced reproduction needs to be considered, since they are important factors affecting the assessment of toxicity, bioaccumulation and ecological quality of the sediments.

EC06A-4
Effect of activated carbon, biochar and compost on the desorption and the biodegradation of low concentrations of phenanthrene sorbed to different soils
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Polycyclic aromatic hydrocarbons (PAHs) are an important class of soil and groundwater pollutants. Often, a large portion of the PAHs are degraded by soil microorganisms within short times (<100 days), and this is often followed by slower degradation resulting in a non-degradable residual fraction. Such a non-degradable residual fraction is very difficult to remove even by intensive biological and chemical methods. This does imply however, that this non-degradable residual fraction is poorly available to organisms and therefore could perhaps be considered as posing a limited risk.
The addition of PAH sorbing amendments to soil leads to reduced freely dissolved concentrations. On the one hand this limits bioavailability and uptake by organisms leading to reduced toxicity, but on the other hand this might also decrease biodegradation. The aim of this study was to characterize the soil amendments activated charcoal (AC), biochar (charcoal) and compost for their ability to reduce the desorption and biodegradation of phenanthrene as a model PAH in three different sandy loam soils (Outfield, RS, or Olsen).
The extent of abiotic desorption of [9-^{14}C]phenanthrene from suspensions made up of soil (either Outfield, RS, or Olsen) plus amendment (either AC, charcoal, or compost) was investigated over a period of 24 days by desorption into an infinite silicone sink. The extent of desorption was then compared to the extent of mineralization (t = 15 d) of phenanthrene sorbed to the soil plus amendment suspensions by *Sphingomonas sp* (DSM 12247). The total amount of phenanthrene desorbed was 6 to 10% for AC, 38 to 44% for charcoal, 87 to 106% for compost, and 95 to 106% for control without any soil amendments after 24 d. This was more than percentage of initial ^{14}C found in the CO_2-trap at experiment completion, i.e., amount mineralized. These ranged between 3.0 to 5.4% for AC, 10.4 to 14.8% for charcoal, 14.9 to 21.8% for compost, and 25.5 to 31.2% for control.
The amounts of phenanthrene mineralized were slightly lower than the maximum amounts that were abiotically desorbed, and indicate that sorption to the soil amendments had a stronger inhibitory effect on mineralization than abiotic desorption. Nevertheless, desorption into an infinite silicone sink might be useful as a tool to estimate the maximal extent of mineralization (and biodegradation) in soils polluted with PAHs.

EC06A-5
The influence of field aging of activated carbon in sediment on PCB sorption in field trials
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Bioavailability of hydrophobic organic contaminants of concern such as polychlorinated biphenyls (PCBs) in sediments is strongly influenced by the nature of contaminant binding. This observation is at the foundation of utilizing the sorption capacity of activated carbon (AC) to control risks posed by sediment-associated contaminants. Monitoring over several years at pilot-scale application sites at Hunters Point, CA and Grasse River, NY USA has demonstrated that AC amendment reduces contaminant bioavailability by controlling both chemical accessibility and activity. One important question is the long-term sustainability of this remediation strategy under field conditions. To further evaluate the sorption effectiveness of AC after prolonged exposure in the field, sorption of freshly spiked and native PCBs to 1) AC aged for 2-2.5 years under field conditions and 2) fresh AC amendments to untreated sediments were compared for sediments collected from both pilot sites. Pore water concentrations and sorption coefficients (K_{AC}) were determined using passive samplers in batch tests. In a separate study, a mass transfer model simulation of the effectiveness of AC amendment

to reduce pore water concentration at Hunters Point was compared to measured values for up to 5 years of field aging. Insights drawn from this modeling effort help to explain results from the sorption study. Values of K_{AC} for field-aged AC were lower than freshly-added AC for spiked PCBs up to a factor of 10, while the effect was less for native PCBs. For both Hunters Point and Grasse River field-aged AC, similarly diminished sorption compared to fresh AC for the spiked PCBs was observed. However, there was a greater decrease in sorption coefficients compared to values for fresh AC for most of the native PCBs in the Grasse River sediment which is likely due to the nearly ten times higher organic carbon content. Mass transfer modeling for Hunters Point indicates that the mass transfer limitations of PCB sorption may change over time in relation to natural organic matter (NOM). The AC does not lose sorption capacity, yet incremental improvements may slow with time. Site characteristics such as the contaminant desorption kinetics and NOM quality and quantity will be important considerations when designing sustainable remediation strategies with AC amendment. Importantly, these studies show that aged amended AC continues to effectively sorb PCBs several years following field application.

EC06A-6

Impact of biochar on the biodegradation and bioavailability of organic contaminants in soil

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The fate of hydrophobic organic contaminants (HOCs) is often controlled by the organic matter content of the soil. However, organic matter may further be mineralised or degraded by microorganisms and eventually release such organic contaminants. In order to enhance remediation of HOCs, biochar, which is a recalcitrant carbon rich product from the pyrolysis of biomass can be utilised. This study investigated the influence of 0%, 0.1%, 0.5% and 1.0% of two different wood waste biochar (BC1 and BC2), and the influence of 0%, 1%, 5% and 10% of BC1 (2 mm and 3-7 mm) on the relationship between microbial mineralisation and hydroxypropyl-β-cyclodextrin (HPCD), $CaCl_2$ and methanol extractions of ^{14}C-naphthalene, phenanthrene and azoxystrobin in soil. The amendment conditions were aged for 0, 18, 36 and 72 days (naphthalene), 0, 35, 70 and 140 days (azoxystrobin), 0 and 40 days (phenanthrene). The total extent of ^{14}C-associated mineralisation was assessed by monitoring ^{14}C-associated mineralisation over 14 days in respirometric assays and compared to HPCD, $CaCl_2$ and methanol extractions. Results showed that BC1 and BC2 amendments showed significant reduction ($p < 0.01$) in extent of ^{14}C-naphthalene mineralisation and extractions compared to 0% BC. Both particle sizes showed significant reduction in extent of ^{14}C-phenanthrene mineralisation and extraction compared to 0%. However, the reduction was greater in 2 mm BC1. There was little or no mineralisation of ^{14}C-azoxystrobin but BC1 and BC2 showed reduction in extractability. Linear correlation between HPCD extractability and total amount mineralised (^{14}C-naphthalene and phenanthrene) revealed very good correlation in all concentrations of biochar amendments BC1 ($r^2 = 0.94$, slope = 0.94, intercept = -0.86) and BC2 ($r^2 = 0.94$, slope = 0.90, intercept = -1.34) for naphthalene and ($r^2 = 0.99$, slope = 1.02, intercept = 0.15) for phenanthrene. Additionally, the $CaCl_2$ and methanol extractions underestimated and overestimated extent of mineralisation respectively. This paper thus suggests that biochar used in this study can reduce the bioaccessibility of HOCs and that HPCD extraction strongly predicts the bioaccessibility of naphthalene and phenanthrene in soils amended with biochar. Thus, biochar may be a useful tool in sorption of pesticides to prevent leaching to ground water. However, the production process, particle size, feedstock and soil properties determine the capability of biochar to adsorb organic contaminants.

EC06B-1

Toxicity and bioavailability of geogenic polycyclic aromatic compounds from coal

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Coals contain native polycyclic aromatic compounds (PAC) in varying amounts depending on coal properties, e.g. origin, coal rank or biological precursor material. As a result of longterm mining activities and usage of coal as an energy source, soils and sediments worldwide can be highly contaminated by unburned coal particles. Most polycyclic aromatic hydrocarbons (PAH) that occurred in coal-rich river floodplain soils were associated with coal particles. Studies on the bioavailability of these compounds come to the conclusion of no or very low bioavailability as a result of coal acting as a very strong sorbent. The highly varying properties of coals including the content of heterocyclic aromatic compounds (NSO-PAC) and PAH were not taken into account. Hence, the aim of the present study is (1) to verify the assumption of no or very low biovailability of coal-derived PAC for different selected coal samples of varying properties and (2) to identify former less known toxic PAC. The results are expected to impact future risk assessment of coal-rich soils and sediments.
Subbituminous (n = 2), bituminous (n = 5) and anthracite (n = 1) coals were used in various contact assays and a bioaccumulation test to assess bioavailability of PAC. Fractionated extracts of the coals were additionally used in bioassays to assess the potential toxicity.
EPA-PAH analysis of the aromatic fraction of the coal extracts revealed highest concentrations in bituminous coal extracts (up to 120.1 mg/kg). Both fractions of all coal extracts (except the anthracite) exhibited dioxin-like activity and cytotoxic effects. The aromatic fractions of all coals (except the anthracite) and the heterocyclic fractions of bituminous coals were mutagenic. Both fractions of all coal extracts (except the anthracite) were toxic in assays in liquid medium for D. rerio, the aromatic fractions were toxic for C. elegans. Results of the contact assays using whole coal particles with the same organisms were different: While the whole coal samples led to no mortality in the fish embryo assay (0 to 2.5%), inhibition of of C. elegans reproduction was 83 to 96% induced by the different coal samples.
We conclude that there is no bioavailabilty of PAC from coals that leads to mortality in the fish embryo assay. Effects in the nematode contact assay are suspected to result from damages caused by the physical properties of the particles.
Further results will be presented.

EC06B-2

Adsorption of organic contaminant from aqueous solution on natural porous material

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In 2000, the European Union adopted the Water Framework Directive (WFD) which project to obtain a good status of water and aquatic system by 2015. Substances posing health risk to human and to the aquatic environment are listed in two categories: substances that should be reduced and substances that should be eliminated. Pharmaceuticals and pesticides are concerned by this regulation project. Particularly hazardous, some of these compounds have tendency to accumulate in living organism where they exert toxic effects.
The wastewater treatment process used currently are not efficient enough to remove all of these compounds and residues of these chemicals are persisting in surface water. During the last years, biological, chemical and physical methods have been developed in order to remove organic contaminant from water. Among these processes (nanofiltration, reverse osmosis, biological degradation, ozonation), sorption is one of the most attractive especially when the adsorbent is low-cost.
In this study, we propose to investigate the adsorption capacity of different natural adsorbents for pharmaceuticals and pesticides. Zeolites and clays are probably the most promising alternatives to high cost adsorbents. Zeolites belong to the family of hydrated aluminosilicates. Their structure is formed with SiO4 and AlO4 tetrahedra confering them a large internal and external surface area and high porosity. Sepiolite is a hydrated silicate of magnesium presenting a fibrous structure. So we have selected three zeolites among the most abundant in nature (clinoptilolite, mordenite and chabazite) and one clay (sepiolite) in order to investigate and compare their adsorption capacity for drugs and pesticides.

EC06B-3

Novel pathways in the adsorption of weak organic acids by black carbon leading to ionization constant shifts on the surface

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Black carbon (BC) is a natural component of soils and sediments, and the use of manufactured BC (activated carbons, biochars) has been proposed as a remediation tool. A fundamental understanding of the adsorption mechanisms of contaminants to BC is a prerequisite to technological control and an accurate evaluation of bioavailability. Although many contaminants are ionic or ionizable over the normal environmental pH range, little attention has been paid to the interaction of such compounds with BC. Here I show examples of weak organic acids that adsorb to BC by unconventional mechanisms. We studied the adsorption to biochars of the allelopathic aromatic acids AA, cinnamic (pKa 4.44) and coumaric (pKa 4.39) and the veterinary antibiotic sulfamethazine SMT (pKa1 2.28, pKa2 7.42; existing as SMT0, SMT+, SMT- or SMT+/-). Extending several units above the pKa, the anionic forms AA- or SMT- adsorb by first undergoing proton exchange of the carboxylate or sulfonamidate group, respectively, with water, liberating hydroxide ion. Proton exchange is followed by adsorption of the corresponding free acid, AA or SMT0. This results, essentially, in a pKa shift of the organic acid on the surface of +5.3 units (AA) or +3.0 units (SMT) relative to the corresponding pKa in water. Since the increase in hydrophobicity is insufficient to compensate for the proton exchange penalty, the driving force for this reaction we believe is the formation of a strong H bond between the organic acid and a surface carboxylate or phenolate group having a comparable pKa. Such “low barrier H bonds”rank among the strongest known in organic chemistry. The bonds are depicted as [RCO2[3DOTS]H[3DOTS]O-surf] and [-SO2N[3DOTS]H[3DOTS]O-surf] where the proton is shared almost evenly between the heteroatoms. At pH values where SMT is protonated, the charged p-aminobenzene group undergoes pi-pielectron donor-acceptor interaction coupled with cation-pi interaction with the pi-electron rich graphene

surface (termed pi+ -pi EDA). At intermediate pH values, the pi+ -pi EDA bond drives a positive shift in the pKa1 of SMT and an increase in the stability constant for the zwitterion SMT+/- on the surface relative to the respective value in water.
We conclude that adsorption of weak organic acids on black carbon surfaces can result in appreciable shift in ionization constants on the surface driven by the formation of strong complexes, such as low barrier H bonds and pi+ -pi EDA interactions.

EC06B-4
Determining sorption of cationic surfactant to organic matters and clay minerals- An application of Ion-Exchange SPME method
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Sorption property is crucial for the mobility of organic compounds in environment and hence is highly relevant to the bioavailability of those chemicals. The freely dissolved is mostly regarded as the bioavailable fraction and often measured by passive sampling techniques based on equilibrium partitioning processes. In this study, a 7-µm polyacylate SPME fiber was successfully used as an Ion-Exchange passive sampler for measuring freely dissolved cationic surfactant. Cationic surfactants are permanently charged organics and have high affinity to negatively charged environmental media (e.g. surface of clays and humics). By employing the SPME method, we studied the sorption behavior of benzyl dimethyl dodecyl ammonium chloride (C12-BAC) to different sorbents, including humic acids, clays minerals and an artificial sediment. By identifying the composition of the artificial sediment and measuring the sorption coefficient of the individual sediment component, the sorption behavior to the whole sediment could be estimated, which was comparable to the data for sediment-only sorption experiment. Whether the sorption of cationic surfactant to field sediment could also be extrapolated via such a method requires further research.

EC06B-5
Walking the tightrope of bioavailability: growth dynamics of PAH degraders on vapour-phase PAH
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Effective biodegradation of soil contaminants requires both adequate environmental conditions and a suitable contaminant bioavailability to degrading microbes. Compound bioavailability however, is 'Janus-faced'; i.e. is essential and likely promoted by the organism for assimilative uptake, whereas, when too high, may lead to toxic effects and provoke avoidance strategies (e.g. tactic responses) of the target organisms. Although the bioaccessible compound pool for both effects may be identical, the exposure of individual organisms to environmental chemicals has opposite consequences and may particularly interfere, when a metabolisable substrate causes toxicity at high bioavailability. Such situation leads to a microbial tightrope walk that is often overlooked in bioremediation studies where one tends to assume that effective pollutant-utilizing bacteria tolerate any exposure to these substrates. The highlights of this paper give evidence of down-gradient, i.e. negative (chemo-)tactic movement of *Pseudomonas putida* (NAH7) away from a NAH point when exposed to vapour phase NAH (surprisingly at even at gaseous concentrations lower than aqueous concentrations that clearly induce chemo-attraction). They secondly elucidate the tightrope walk of substrate bioavailability for assimilative growth and growth inhibition of strain PpG7 in vapour-phase NAPH gradients: Microcosm experiments revealed that high cell densities increased growth rates close (< 2 cm) to the NAH source, whereas intercellular competition for NAH decreased growth rates and biomass at larger distances despite the high NAH gas phase diffusivity. Finally, such varying growth kinetics is explained by a combination of bioavailability restrictions and NAH-based inhibition. To account for this balance, a novel, integrated 'Best-equation' describing microbial growth influenced by both substrate availability and inhibition is presented. Our work clearly demonstrates the importance of bacteria to degrade vapour-phase compounds and to influence vapour-phase PAH concentration gradients even at the centimeter-scale. It further underlines the importance of high active biomass and concomitant effective reduction of their exposure to inhibitory substrates in order to create environments favourable for survival, which may influence the exposure dynamics and ecology of entire microbial communities and hence actively shape environments beneficial for enhanced biodegradation.

EC06B-6
Contribution of microbial biomass to non-extractable residue formation from an organic contaminant
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Biodegradation of pesticides in soil is actually understood as their transformation into various metabolites, microbial biomass, mineralisation products and non-extractable residues (NER). NER are believed to mainly consist of hazardous parent compounds or primary metabolites sorbed to or sequestered by soil organic matter (SOM). Up to date, however, their chemical composition remains still unclear. This is because numerous studies on NER formation are limited to quantitative analyses in soils or to simple humic acids-contaminant systems. During biodegradation of organic contaminants, the C is used by microorganisms for their biomass synthesis. After cell death and lysis, biomass components are stabilised in SOM ultimately forming harmless biogenic residues. We investigated the formation of biogenic residues during biodegradation of 13C-labelled herbicide (2,4-dichlorophenoxyacetic acid) in soil over 64 days. We prove for the first time that nearly all NER formed from this readily biodegradable herbicide in soil contained only non-hazardous microbial biomass components. Therefore, for the proper assessment of the potential risks of a target contaminant in soil to environment it is necessary to consider a possible biogenic origin of NER in the mass balances of contaminants in soils.

EM01 - Collection and use of monitoring data for environmental risk assessment of chemicals

EM01A-1
Update on the global monitoring plan (GMP) in the UNEP Stockholm Convention on persistent organic pollutants (POPs), technical guidance, data analysis, modeling, assessment and workplan
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The GMP established under Article 16 of the Stockholm Convention on POPs (SC) has been active since 2004 and produced a first global assessment in 2009. It is currently revising the technical guidelines, an extensive document describing recommended methods and procedures to monitor POPs in the GMP core media, air and human tissues (milk, serum) . The work under the GMP has identified the relevance of modeling long range atmospheric transport including meteorological and climatic variability to understand better the effects of a changing climate on POPs . Important results have been published in the United Nations Economic Commission for Europe's (UNECE's) report on Hemispheric Transport of Air Pollution (HTAP) 2010 Part C and the United Nations Environment Programme (UNEP)/ Arctic Monitoring and Assessment Programme (AMAP) report of "Climate Change and POPs: Predicting the Impacts" Challenges for future work include long term stability and coordination of the networks, the analytical and data quality assurance/quality control (QA/QC), data management and archiving, the development and use of models dealing with atmospheric and marine long range transport, as well as modeling ecological and physiological pathways and time lags in exposure leading to health impacts. This paper describes the main results and identified challenges that will define the future work of the GMP, an international cooperative knowledge building process focused on the fate of POPs released into the environment and the possible ways to decrease or eliminate them.

EM01A-2
Adapating monitoring strategy to the contaminant source characteristic - chromium in the upper Dunajec River watershed
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Application of the monitoring data instead of estimated or predicted values for contaminant emissions and concentrations is crucial for environmental risk assessment. Therefore, representativeness and reliability of these data should be assured. Enhanced knowledge about the temporal and spatial patterns of contaminant distribution improves effectiveness of monitoring data collection. Since these patterns are not uniform in aquatic environments carefully designed sampling regime should be considered for different types of aquatic systems, especially in the operational monitoring under WDF. A 19-week continuous water sampling in the upper Dunajec river watershed (Southern Poland) has proved that chromium contamination in this river, originating from the local tanneries, is variable and discontinuous. To compare two monitoring strategies instantaneous (discrete) and integrated (composed) samples were collected. Sampling was performed in three sites, encompassing the local impoundment reservoir system, which allowed also for estimations of chromium load introduced into the reservoir. Discrete samples were collect weekly by hand, while integrated samples were collected using automatic water samplers (ISCO) in the time-integrating mode. This mode rather than discharge proportional mode was chosen for a better comparison with the discrete sampling. To assess impact of monitoring strategy on load computations dissolved and particulate Cr loads in instantaneous and integrated samples were estimated using averaging calculation methods. Study results showed that discrete sampling can lead to an underestimation of chromium contamination level and load, especially when illegal/unexpected discharges occur in the watershed. From chromium load computations based on results for both types of samples we also concluded that integrated sampling produces more reliable data, with acceptable range of estimated errors. Instantaneous sampling, even if frequent, should not be used for contaminant budget calculations in the localities were strong variability of contamination is anticipated.

EM01A-3
Factors influencing the quality of river monitoring data used for environmental risk assessment of particulate/hydrophobic chemicals

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From 2000 to 2009, about 300 chemicals were analysed in water, bed sediment and suspended particulate matter (SPM) at 17 sites throughout the Rhône river network by the regional water authority, providing a very rich and useful dataset. This work focuses on factors influencing the quality of this database. Few studies have been carried out on the analysis of hydrophobic organic contaminants and metals associated with SPM, we thus favoured critical review of these data. Data processing revealed several issues that can weaken the dataset quality. While the constancy of analytical methods is of utmost importance, data processing highlighted major temporal discontinuity for some chemical concentrations, either due to changes of the analytical methods, or due to inappropriate or shifting quantification limits. Furthermore, despite the fact that this dataset refers to a river network, no chemical concentrations were recorded during flood events, biasing any annual average concentration evaluation, especially for contaminants associated with SPM. Thereby, the sampling frequency or strategy should be carefully chosen in order to obtain information on potential variations of concentrations linked to the river hydrological cycle. Finally, sampling methods should be characterised and compared. In a recent study of the Rhone Sediment Observatory we focused on the comparison of different SPM sampling techniques. A difference in the particle size distribution of SPM sampled with the various techniques was noticed, which could have an effect on the concentrations of contaminants associated with SPM. To conclude, this study revealed that factors such as analytical methods, sampling frequency and sampling methods impact concentrations or annual average concentrations of chemicals in SPM, bed sediment and water, and thus impact the monitoring dataset quality. Consequently, the outcome of an environmental risk assessment based on such a monitoring dataset might be significantly affected. Interlaboratory comparison tests, quality controls, as well as a complete documentation on sampling and analytical methods are among possible solutions to build relevant datasets of chemical concentrations in rivers.

EM01A-4
Making management decisions with imperfect data: assessing potential aquatic metal risks with biotic ligand models
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Accounting for bioavailability, through the use of sophisticated Biotic Ligand Models (BLMs), represents the most technically robust method assessing potential metal risks in the freshwater aquatic environment. New user-friendly BLMs (ufBLM) are now available to facilitate their regulatory use. These new models are based on the outputs of the more sophisticated BLMs but require data on fewer water physicochemical parameters to run (i.e. limited to just pH, calcium and dissolved organic carbon). However, there remain obstacles to using the tools, specifically the lack of site-specific physicochemical input data. Absence of required input data means the models cannot be run. Yet, while not always starting with the perfect dataset for all sites of interest, it is rarely the situation that there is a complete absence of "fit of purpose"input data. There are several ways by which input data gaps can be filled in a robust, precautionary, manner to deliver a screening level assessment which can then be used to develop focussed monitoring programmes, identify sensitive sites and broadly characterise risks. This presentation will give an example of how, through the use of imperfect data, management decisions can be made in relation to the assessment of potential aquatic risks of metals.

EM01A-5
Mapping the chemical environment of London: the London earth project
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The British Geological Survey has been involved in a number of programmes to systematically map the concentrations of chemical elements in the surface environment of the United Kingdom. These include both European-wide EuroGeoSurveys' projects (e.g. FOREGS geochemical atlas and GEMAS metals in agricultural soils) and regional projects in rural and urban areas of England and Scotland. In May 2010 the results for the largest urban mapping survey undertaken, 'London Earth', were released. Soils were sampled at a density of four samples per kilometre square from the Greater London Authority Area. This represents one of the world's largest systematic geochemical mapping exercises in an urban area. Over 6,400 topsoil samples were determined for more than 50 elements by X-ray fluorescence spectrometry (XRFS) and other soil parameters such as pH and loss on ignition were determined.
Since the publication of the results further analyses have been done on subsets of the samples including mercury (Hg) and organic contaminant studies, profile lines mapping the occurrence of Au and PGEs (platinum group elements), and bioaccessibility studies. Applications of the London Earth data to environment and health issues will be presented.

EM01A-6
Active local sources of PCBs in the Arctic
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Studies of PCBs in local sources, including building materials such as paint and concrete, small electrical capacitors, and local surface soil were initiated on Svalbard, Norway in 2007. Previous to these studies increasing levels of various pollutants in marine sediments outside specific settlements were discovered, suggesting an active, local source to PCBs.
During 2007-2010 more than 1100 single samples of paint, concrete, soil and small capacitors from 12 different active and abandoned settlements were collected. 78 soil samples from 24 background sites were also collected. The exterior paint in the settlements can be regarded as a primary source of PCBs. In a dry and extreme climate such as exists on Svalbard, paint will eventually flake off and fall to the ground. The settlement soil can then be regarded as a secondary source of PCBs, which can spread the pollution to terrestial or marine ecosystems by wind and/or water erosion. The background sites will be affected by a mixture of long-range transported PCBs and PCBs from local sources.
This work shows active, local sources to PCBs in the Arctic. The levels of PCBs in the primary sources are up to permille levels and the risk of dispersion is evident. These finds should be taken into account in the ongoing discussion of local sources versus and long-range transport sources. From an administrative point of view, this gives an opportunity to actually take care of the pollution sources before they are spread to the environment.
In this presentation the work done at Svalbard will be emphasized, but also other similar work will be shortly reviewed.

EM01B-1
GEMAS: Geochemical mapping of agricultural and grazing land soils at the European and national scales
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Geochemical mapping of agricultural and grazing land soils (GEMAS) is a cooperative project between the Geochemistry Expert Group of EuroGeoSurveys (EGS) and Eurometaux. REACH specifies that industry must prove that it can produce and use its substances safely. During 2008 more than 4000 samples of arable (0-20 cm) and grazing (0-10 cm) land soil were collected at a density of 1 site per 2500 km2 from 33 European countries. The sample density is based on previous experience of the Geological Surveys with comparable projects. The < 2mm-fractions of all samples were analysed for 52 chemical elements in an aqua regia (AR) extraction, 41 elements by XRF and soil properties like CEC, TOC, pH (CaCl2) following tight external quality control procedures. Natural variation is large (3 - 4 orders of magnitude for most elements) and results can be reliably mapped. Distribution maps of all parameters and elements are produced and demonstrate that the chosen scale is fit for purpose. The data allow the study of the spatial distribution of chemical elements and soil properties at the European scale, and better understanding of processes driving the observed patterns. The distribution patterns of selected elements will be discussed based at the European and national scale maps (e.g., Germany, Ukraine). All elements and parameters display clear geographical distribution patterns that can be linked to a variety of natural features, geology and climate playing a key role. Many elements show substantially lower concentrations in Northern than in Southern European soils. The project will provide background values for a large variety of elements in agricultural and grazing land soils at the European scale.

EM01B-2
Metal bioaccessibility in Canadian soils: using the North American soil geochemical landscapes project
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The North American Soils Geochemical Landscapes Project (NASGLP), a collaborative effort among the US Geological Survey (USGS), the Geological Survey of Canada (GSC) and the Mexican Geological Survey, was initiated to provide a soil geochemical database for a broad-based group of users in the field of environment and human health. This Tri-national Survey will ultimately produce a database of the regional natural-occurring differences in concentrations and physicochemical characteristics of soil which can be used to assess background conditions and identify anthropogenic impacts. The survey is based on low-density sampling (within a 40 km by 40 km grid) yielding 13,487 sites across North America. Soil sampling and analysis in Canada was initiated by the GSC in partnership with other provincial and federal agencies including Health

Canada in 2004. Analytical parameters included total metals, pH, total organic carbon, inorganic carbon and loss on ignition. A sub-set of the samples collected in Canada from the surface (0-5 cm, referred to as the public health layer) and the C horizons were analyzed for metal bioaccessibility using a simplified physiologically based extraction test as a surrogate for bioavailability. Results will be presented on the bioaccessibility of naturally-occurring metals in the soil public health layer and the C horizon, along with relationships of bioaccessibility with soil geochemical data. The data obtained indicated some differences in mean elemental bioaccessibility between the public health layer (0 - 5 cm) and the C horizon (parent material) for each province. There were also some statistically significant relationships between metals bioaccessibility and soil pH, total organic carbon and loss on ignition for a sub-set of the samples. Comparison of the elemental bioaccessibility in the natural background soils to literature information on bioaccessibility from contaminated sites (e.g., mine sites) in Canada will also be shown, along with how the data might influence derivation of generic soil quality guidelines in Canada.

EM01B-3

Quality assurance in the GEMAS project and results of the connected proficiency test

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Quality assurance is one of the keystones to the success of any European scale geochemical mapping project requiring harmonized data. Therefore a set of stringent quality control (QC) measures were introduced into the analytical program of the GEMAS (geochemical mapping of agricultural and grazing land soils) project including field duplicates, analytical duplicates, insertion of two project standards (Ap and Gr) as internal references between true samples, and randomization of all samples prior to analysis. X-Charts proved to be helpful in immediately solving some technical problems as exchange samples or time trends. The within-lab-reproducibility of QC data for the standards Ap and Gr is generally good, but strongly dependent on the analyte. The nested design of duplicate field and duplicate analytical samples allowed carrying out a statistical analysis of variance (ANOVA) to identify the relative contributions of regional, sampling and analytical variances. The ANOVA demonstrates that the technical variability is low enough to detect regional differences in geochemical maps.
Additionally a proficiency test (PT) based on the project standards was performed in 2011 to check the comparability of individual national laboratory data with the data used for mapping, and to confirm the trueness of the mapping data. In total 21 institutions from 16 countries submitted analytical data, including the mean values from QC analyses as "normal participants". Evaluation of data and laboratory assessment was done using robust statistics, limited standard deviations and zu scores. Results are presented for between-lab-reproducibilities, Horwitz ratios as indicator for PT performance, and inter-method discrepancies between analytical methods and sample preparation techniques. The assessment of the QC data for the standards Ap and Gr in this PT confirms the trueness of total element contents and most AR contents.

EM01B-4

Comparison of XRF and Aqua Regia data from agricultural soil in Europe: results from the GEMAS project

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The comparison of analytical results from aqua regia (AR) and X-ray fluorescence spectroscopy (XRF) can provide information on soil processes controlling the element distribution in soil. The GEMAS (GEochemical Mapping of Agricultural and grazing land Soils) agricultural soil database, consisting of 2 x ca. 2100 samples spread evenly over 33 European countries, is used for this comparison. The GEMAS project was designed to fulfil REACH (Registration, Evaluation and Authorisation of Chemicals) requirements (e.g., land use, sampling depth). It minimised critical error sources by standardisation of sampling procedures, sample preparation and analysis. Analyses for the same suite of elements and parameters were carried out in the same laboratory under strict quality control procedures. Sample preparation has been conducted at the laboratory of the Geological Survey of the Slovak Republic, AR analyses were carried out at ACME Labs, and XRF analyses at the Federal Institute for Geosciences and Natural Resources, Germany.
Element recovery by AR is very different, ranging from <1% (e.g. Na, Zr) to > 80% (e.g. Mn, P, Co). Recovery is controlled by mineralogy of the parent material, but geographic and climatic factors and the weathering history of the soils are also important. Nonetheless, even the very low recovery elements show wide ranges of variation and spatial patterns that are affected by other factors than soil parent material. For many elements soil pH have a clear influence on AR extractability: under acidic soil conditions almost all elements tend to be leached and their extractability is generally low. It progressively increases with increasing pH and is highest in the pH range 7-8. Critical is the clay content of the soil that almost for all elements correspond to higher extractability with increasing clay abundance. Also other factors such as organic matter content of soil, Fe and Mn occurrence are important for certain elements or in selected areas.
This work illustrates that there are significant differences in the extractability of elements from soils and addresses important influencing factors. Consequently, soil risk assessments for elements or metals should be made using effects and exposure values, based on the same extraction method.

EM01B-5

Use of monitoring data for environmental risk assessment of metals in soil

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A sound risk assessment for trace elements in soil preferentially takes into account the spatial variation in natural background concentration and in metal bioavailability. Data availability for both aspects differs largely across different countries or regions, hampering a consistent approach on a large (e.g. regional or continental) scale. It is therefore often difficult to compare results for regional risk assessments among different countries. The GEMAS (geochemical mapping of agricultural and grazing land soil) project provides good quality and comparable exposure data of metals in agricultural and grazing land soil at the European scale. In addition soil properties known to influence the bioavailability and toxicity of metals (and other elements) were determined in the same soils. The GEMAS project was carried out by the EuroGeoSurveys (EGS) Geochemistry Expert Group in cooperation with Eurometaux and the aim of this project was to produce harmonised data with respect to the spatial scale (sampling density), analytical methodology and land-use (comparable level of diffuse emissions). The results allow a consistent risk characterisation across Europe and country"specific results can be directly compared. The data also provide a strong basis for taking into account the spatial variability of both exposure (metal concentrations) and effect concentrations (considering bioavailability through variation in soil properties) in a risk assessment for metals in soils and therefore avoid the need for (worst-case) assumptions on both aspects. The use of these GEMAS monitoring data for regional and local risk assessments of metals in soil will be discussed based on examples for several metals.

EM02A - Fate and exposure modelling

EM02A-1

Estimating and evaluating cumulative human exposures to ubiquitous pollutants: integration of outdoor, food web, and indoor fate models with exposure biomarkers

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Widespread observations of environmental contaminants in house dust, food, vegetation, soil, animals, and human tissue have motivated research on better understanding of exposure pathways for a broad range of contaminants over indoor, urban, regional, continental, and global scales. This presentation addresses how fate models at different levels of geographic scale combined with environmental and biomarker measurements can be used to interpret and predict cumulative human exposure from multiple pollutants and emissions sources. We consider three case studies to explore the insights gained by simple but informative integration strategies. First we look at regional scale pesticide exposures in a farming community and compare local contributions to exposure from pesticide use on fields with contributions from food produced within and outside the region. For this study we have a reliable source data and robust biomarker data. Here we find that the integration of outdoor and indoor fate models is key to tracking overall intake. We next look at cumulative exposures to combustion-produced polycyclic aromatic hydrocarbons across ~3000 counties in the United States for which emissions data are available. For this case the variations of county emissions track well with the variations in national-scale biomarker data. But there are clear indications that cumulative intake is dominated by food and indoor exposures. Finally, we look at commercial buildings with filtered and re-circulated air to assess how indoor exposures to particle-bound semi-volatile organic contaminants (SVOCs) are impacted by the level of air recirculation. For this case the reduction of SVOC exposure indoors relative to outdoor levels is sensitive to both the octanol-air partition coefficient and the fraction of air re-circulated. These three cases reveal that the relative contributions to cumulative pollutant intake via different exposure pathways depend on (a) persistence of chemicals at different levels of integration (regional, urban-scale, food-web, indoors), (b) basic chemical properties, (c) the retention of chemicals in food webs, and (d) the retention of chemicals by indoor surfaces.

EM02A-2

Reducing empirical data need in fate and exposure models by overall (scaling) principles

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Environmental chemistry and toxicology face the immense challenge of protecting thousands of species from thousands of substances released into thousands of different landscapes. Empirical studies are limited because of financial, practical, ethical and time-space restrictions. To cover all relevant cases, fate and exposure models have been developed. Yet, such models too, tend to become data hungry because of parameterization. Properties of chemicals have extensively been used to extrapolate knowledge, allowing one to estimate default values for parameters in the absence of experimental data. The objective of the present paper is to extend this approach to species traits and landscape characteristic. In particular, we aim to identify similarities and differences in size scaling in the various disciplines involved, i.e. hydrology, chemistry, biology and technology.
Confining ourselves to lakes and organisms, we will show similarities and differences in the turnover of water, energy and chemicals. Both data and theory suggest that inflow in lakes scales geometrically while inflow in organisms (ventilation, consumption etc.) increases allometrically.
Understanding the overall principles determining the cycling of water and biomass helps us to predict the fate and accumulation more accurately. Rather than choosing a "typical"value for run-off in fate models, we might choose the average and standard-deviations noted for catchments of a certain size. Instead of picking an uptake rate constant for accumulation from a more or less related species, we can now estimate the kinetics in poorly investigated specimens from their body size. In addition, overall principles as size scaling open up opportunities to design generic models.
While we focus on the approach for size scaling, other variables can help to refine explanations. Estimations can be improved by adding temperature as an explaining variable, allowing one to distinguish between temperate and tropical regions or between cold-blooded and warm-blooded animals. In addition, size scaling can also be applied to other parts of the environmental cause-effect chain. As an example for emissions of carbon dioxide and polycyclic aromatic hydrocarbon, fuel use by engines scaled similarly to running organisms. At the same time turnover and species abundance (size spectra) in ecosystems can be related to environmental stressors.

EM02A-3
Not PBT, but LRTP - Identification of substances with possible very high concern
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In the European chemicals legislation REACH, a substance is considered of very high concern if it is persistent, bioaccumulative and toxic (PBT). A substance's long range transport potential (LRTP) and the subsequent hazard to remote regions are not explicitely included but can give cause for serious concern. To identify compounds, which would not be classified as PBT substances but are prone to LRTP (non-PBT-L substances), we screened the Canadian Domestic Substance List (CDSL), which contains 22,438 compounds in use on the Canadian market.
In a first step, the CDSL was searched for organic compounds which are to more than 95% in the neutral form at an environmentally relevant pH range. The identified compounds were run through EPI Suite v4.0 such that substance properties were quantified by the implemented estimation methods. Next, the list was edited in two ways to identify compounds with LRTP. On the one hand, the half-life criterion for long-range transport in air as defined in the Stockholm Convention was applied (half-life in air > 2 days). On the other hand, all compounds were simulated with the multimedia model ELPOS v2, which calculates relative indicators for persistence and LRTP (Matthies et al. 2009). Applying the half-life criterion of 2 days in air as an indicator for LRTP, we identified 621 substances, which are prone to LRT; 594 of these are not PBT. In contrast, investigations with ELPOS lead to a shorter list of 188 substances which are not PBT but show LRTP (non-PBT-L substances). All 188 non-PBT-L compounds are persistent, but not bioaccumulative. However, in order to decide if these substances are of very high concern the list was compared with potential Arctic contaminants identified by Muir and Howard (2006) and by Brown and Wania (2008), respectively. This combination of different screening methods allows to focus on a subset of eleven organic substances, which are not PBT but identified to be charaterized by LRTP An additional literature research on substance properties elucidates possible hints on the reliability of the screening procedure.

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EM02A-4
New read-across model to estimate the BCF for fish from similar chemicals
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The bioconcentration factor (BCF) is a key property to assess the bioaccumulation of organic chemicals. Existing estimation models relate the BCF for fish to the octanol/water partition coefficient. Available experimental data from literature have been collected and thoroughly validated, resulting in a high quality data set of BCF for fish covering 1000 organic chemicals.
Theread-across approach calculates the BCF from similar compounds by a similarity weighted averaging. The selection of the similar compounds and the numerical calculation of the similarity weights are achieved by atom centred fragments (ACF).
The similarity threshold to consider reference compounds can be fine-tuned to balance between accuracy and generality. With the default threshold, the model yields valid results for ca. ¾ of the chemicals. The statistics clearly demonstrate the reliability of the new approach. The model reliability is further investigated by inspecting the value diversity and the molecule size ranges for the selected compounds. A relationship between the estimation errors and these ranges can be shown.
The model performance roughly compares to the literature models. The errors of the new approach are not correlated individually per compound to the respective errors of the other models. However, it can be shown that generally agreement of the prediction results is related to small estimation errors. This offers the opportunity to apply a suite of models for consensus modelling.
The new model as well as the studied literature models is already implemented in the software system ChemProp. The software offers uncertainty estimation for the result in addition. ChemProp is publicly available for free, based on a bilateral license agreement.
The development of this method has been supported by the EU Integrated Project OSIRIS (Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Test Information, contract No. 037017). A part of the data was compiled within the former EU project CAESAR (Computer Assisted Evaluation of industrial chemical Substances According to Regulations, contract No. 022674).

EM02A-5
Modelling the impact of dietary transitions on human exposure to bioaccumulating organic contaminants
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By combining models of a contaminant's fate in the physical environment with models of bioaccumulation through the food chain, it is now possible to describe mechanistically and dynamically the entire sequence of events linking emissions and body burden in humans. Here we rely on such a model combination to examine the role of dietary transitions on human contaminant levels. Concentrations of persistent organic pollutants (POPs) in Inuit populations have been observed to decrease over the last decade or so. Are these decreases an indication of the success of international efforts to reduce the emissions of POPs globally or did they occur because the diet of the Inuit has shifted away from traditional food items toward store-bought food? It is assumed that the diet of the Inuit is composed of regionally harvested traditional food and of food imported from the South. The global contaminant fate model is used to calculate time trends of contamination in the physical environment of the Arctic and the Northern temperate zone over the past 100 years. Using the calculated contamination time trends in the Arctic environment as input, the contamination of the traditional country food is calculated using the ACC-Human bioaccumulation model The contamination of the imported food is similarly calculated using ACC-human and the calculated contamination time trend in the temperate environment. Longitudinal body burden age trends of a number of individuals representing different sections of Inuit society are simulated: Those maintaining a predominantly traditional diet throughout their life-time, and others who underwent dietary transitions at different times, ages, and to a different extent. By combining multiple longitudinal body burden age trends calculated for individuals born in different years, cross-sectional body burden age trends can be generated and compared with those obtained from cross-sectional human biomonitoring studies. The model predicted concentrations of POPs decline over the past decade both in Individuals who maintained a traditional diet as well as those that increasingly relied on store bought food from the South for sustenance. The latter group is predicted to have experienced a faster decrease in exposure. Transitions in the diet from traditional to store-bought food may contribute at least as much to declines in contaminant concentrations in Inuit as declines in environmental contaminant concentrations.

EM02A-6
Modelling approach to estimating the source strength of cyclic volatile methylsiloxanes from the city of Zurich, Switzerland
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The cyclic volatile methylsiloxanes (cVMS), octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6), are widely used in personal care products. Given their high volatility resulting in a large fraction of the used amounts being emitted to the atmosphere, their persistence in the environment, and their potential for long-range transport, it is important to improve knowledge about emission sources to provide a basis for future environmental fate modelling and for the risk assessment and management of cVMS. In this study, we present a novel approach to estimating the urban source strength of cVMS by employing a multimedia mass balance model in combination with measured cVMS concentrations in air, and apply it to D4, D5 and D6.
Diel variations of ambient air concentrations of D4, D5 and D6 were measured at two sites in Zurich, Switzerland, in winter 2011. One site was located in the valley near the city center and the other site was on a nearby hill. A period with a temperature inversion was chosen for the sampling campaign in order to investigate the enrichment of cVMS in the boundary layer. A multimedia mass balance model was developed and employed to (i) explain the concentration trends observed at both sites in terms of dominant processes and (ii) to estimate urban source strengths. Choosing the emission rate as an adjustable model parameter made it possible to estimate the source strength based on empirical data gathered during the study or available in literature. Key parameters include the measured cVMS concentrations, inversion layer height, temperature, wind speed, and physicochemical substance properties of D4, D5 and D6.
Levels of D4, D5 and D6 in air in the city center were 14-110, 67-450 and 7-57 ng/m^3, respectively. Concentrations on the hill were lower (3-49, 29-110, 5-12 ng/m^3). There was an increase in the concentrations by about a factor of three in the city during our sampling period, and this could be attributed by the model to the enrichment of the chemicals in the boundary layer. Boundary layer height, which varied considerably throughout the sampling campaign, was determined to be the dominating factor. Degradation by OH radicals and deposition were of less importance. For D5, our estimated source strength compares well with estimated emission rates derived from studies on emission rates from individual buildings or emissions estimates based on market information.

EM02B-1

Scenario-study of the expected cumulative impact of European Union environmental legislation on the aquatic discharges from land - an example with three priority substances

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In order to support the implementation of the Marine Strategy Framework Directive, it was carried out an evaluation of the impact of EU environmental legislation on the quality of the marine environment, with reference to discharges to the European seas. The assessment focused on current trends and future options for reduction of inland-based chemical emissions. The evaluation was intended to check a methodology developed at JRC-IES (MAPPE-Europe model) for the identification of hot-spots in Europe and its capability to estimate the likely chemical loads to European coastal waters under different scenarios.
The work describes outcome of scenario analyses up to 2020 for Lindane (gamma-HCH), Trifluralin and Perfluorooctane sulfonate (PFOS) taken as pilot substances. We assumed different types of legislative measures (business as usual, ban, phase out, etc.) or aimed at specific targets (total or disaggregated load to European seas and possible "cleaning-up"of soil in Europe). When considering the outputs from the scenarios, it is important to take into account the fact that the project aimed to test the applicability of the modelling platforms and that, since limited datasets were used and certain assumptions had to be made, the outputs should be regarded as approximative.
For Lindane, the model estimated sea load of 745 tons per 1995, based on the official emission data provided by EMEP, appears to be reduced by 98.3% in 2005, ten years after the start of the EU regulations for gamma-HCH. Besides, under the BAU scenario, a Lindane sea load of ca.12.5 tons per year should be expected. The trend and ban scenarios support, respectively, a reduction of the load to the European seas in 2020 by 74% and 95% when compared to the BAU estimate.
Looking at Trifluralin, according to the BAU scenario, an annual load of ca.61.7 tones is estimated in 2020. However, this is an overestimation of sea load, because the aggregated emission data of EUROSTAT for the agriculture use of the entire group of dinitroaniline herbicides for EU25 have been considered as model input data. The ban scenario forecasts ca. 0.07 t/y, a negligible level that, due to degradation in soil,in practice eliminates the concern about loads of Trifluralin to European seas.
Considering PFOS under BAU scenario conditions, the total sea load from European countries is estimated to be 5.8 tons per year. The model forecasts approximately a half of this amount when a 50% reduction of emissions takes place.

EM02B-2

Modelling assessment of climate change-induced effects on the fate of POPs in the Baltic Sea region

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The recently released report of the UNEP/AMAP Expert Group stresses that global climate change may cause redistribution of some legacy persistent organic pollutants (POPs) and possibly counteract the efforts which have been made to reduce releases of POPs under the Stockholm Convention. In the present study we assess how predictions of the future variation in three climate change parameters, temperature, wind speed and precipitation, affect the environmental fate and distribution of POPs in the Baltic Sea region using the POPCYCLING model. The model was applied to the entire range of possible perfectly persistent POPs within the following property ranges; $0 < \log K_{OW} < 12$, $5 < \log K_{OA} < 14$, and $-6 < \log K_{AW} < 4$. Perfect persistence was assumed for all the POPs to represent a bounding scenario in which the effects of redistribution of POPs due to a changing climate are maximized. Two greenhouse gas emission scenarios were adopted which are referred to as A2 and B2 representing high and low CO_2 emissions, respectively. A baseline ("present day") scenario was adopted as a control scenario for comparison with the future climate change scenarios. Quotients were calculated between predicted POP concentrations in air based on the A2 or B2 climate change scenarios versus the baseline scenario. The results show that the more extreme climate change scenario (i.e. the A2 scenario) causes relatively more pronounced changes in the modelled air concentrations irrespective of to which media the emissions are assumed to occur. Climate change-induced variations in the modelled concentrations in air vary with changing emission mode. Greater changes in modelled concentrations are observed when emission is assumed to occur to the water or soil compartment, i.e., the quotients between predicted air concentrations under the A2 or B2 scenario versus the baseline scenario range from 0.6 to 2.0, but the quotients range from 0.6 to 1.7 when emission is assumed to occur to the air. Our results thus indicate that the influence of climate change on the concentrations of POPs in air is strongly dependent on the partitioning properties and emission characteristics of the substances, and that although climate change-induced effects on partitioning of POPs may work to counteract emission reductions efforts for some POPs, in other cases climate change may hasten the removal of the POPs from the atmosphere.

EM02B-3

Persistent organic pollutants in the North Sea in the 21st century: simulations with a combined hydrodynamic and fate and transport ocean model

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The fate and cycling of two selected persistent organic pollutants (POPs), PCB 153 and gamma-HCH in the North Sea in the 21st century is modelled with a combined hydrodynamic and Fate and Transport Ocean Model (FANTOM). Large amounts of POPs enter the North Sea system through atmospheric deposition and river inputs, with additional contributions coming from bottom sediments and adjacent seas. To investigate the impact of climate variability on POPs in the North Sea in the 21st century, future scenario model runs for three 10 year periods to the year 2100 are performed. Based on the IPCC A1B (SRES) scenario for the 21st century, our hydrodynamic model results show that average water temperature in the North Sea increases by 2 °C, while salinity is reduced by about 0.59 psu. In the atmosphere, temperatures at 2m increase by 2.38 °C. For wind speeds (10 m), mean values do not change significantly but increases in local maxima values and duration of gale wind conditions are significant. It is the effect of these changes on POPs that are analysed. Hydrodynamic variables are calculated with the Hamburg Shelf Ocean Model HAMSOM model and POPs processes are calculated with the FANTOM model. Since estimates of future concentration levels of POPs in the atmosphere, oceans and rivers are not available, our approach was to reutilise 2005 values in the ocean, atmosphere and rivers for every year of the simulations, while using the (final) result of our 1996 - 2005 runs as the initial condition for all three future runs. In this way, we attribute differences between the three 10-year simulations to climate change only. A cursory look at the results show that for gamma-HCH total mass is highest in summer in both water and sediment with a clear seasonal cycle; total mass in water remains fairly steady for each of the 10-year runs but decreases in each of the future runs relative to the first; total mass in sediment increases over time with future runs increasing faster. For PCB 153, total mass in water remains steady while decreasing linearly in sediment; total mass is greatest in water and least in sediment during the winter months when winter storms cause resuspension from the sediment into the water column (this process is also seen in gamma-HCH). Presently, we investigate to which components of the climate system these changes can be attributed. It appears the North Sea acts as a sink for gamma-HCH but as a source to the atmosphere for PCB 153.

EM02B-4

Geo-referenced exposure modelling of pharmaceuticals in river basins supports selection of mitigation strategies

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The geo-referenced model GREAT-ER can be used to simulate expected concentrations of pharmaceutically active chemicals (PhaC) in surface water. The model was amended by features for simple menu-controlled definition of measures to reduce PhAc emissions into surface water. Scenario results can be displayed as concentration ratios against a reference scenario. Predictions of spatially resolved concentrations of the antibiotic Clarithromycin in the Main river basin (Germany) are presented to demonstrate how the model can support the selection of promising mitigation strategies. A reference scenario was prepared representing the current state of the contamination with Clarithromycin. Two different scenarios were selected to demonstrate the feature of the model for a priori evaluations of the effect of measures: (i) a local scenario in which two of the larger WWTPs discharging directly into river Main were arbitrarily selected to be equipped with an additional ozonation treatment step with 90% removal efficiency for Clarithromycin; and (ii) regional scenario for the eastern part of the Main catchment in which we assumed that at least 10% less consumption of Clarithromycin can be

achieved in the area due to a shift in prescription and consumption behavior.
The effect of the scenarios is evaluated by comparison of the concentration profiles of Clarithromycin along River Main. The effect of the local technical measure at the upstream WWTP is evident from much lower simulated concentrations. The additional ozonation at another WWTP more downstream only leads to a small decrease in concentration. The regional measure proved to be much less effective in the upstream part, but results in almost the same improvement more downstream. From the simulation data at the mouth of river Main into the Rhine, it can be seen that both measures obviously removed only a small fraction of the total emission in the catchment. This is an effect of the overlying multi-point emissions from the several hundred wastewater treatment plants of which only a small number have been included in the respective measures. Concluding, the model allows for the identification of local "hot spots"and also an a priori evaluation of potential mitigation strategies. The analysis given shows that for pharmaceuticals sustainable overall reduction of surface water concentrations is hardly to be achieved by local measures, even if some of the largest WWTPs are technically refitted.

EM02B-5

Spatiotemporal exposure assessment of pesticides in flowing waters - results for predicted environmental concentrations in some brooks in Germany

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In 2011 the "Georisk"- project of the German Federal Environmental Agency was finalised. Objective of this project was to form the scientific basis for an integration of more realistic landscape based scenarios into the process of pesticide registration. Here, results of spatiotemporal simulations of PECtwa in flowing water systems are presented. The objective of the simulations was to predict initial environmental concentrations in flowing water bodies resulting from spray drift entries. Based on this the downstream development of these concentrations over space and time with regard to dispersion processes was simulated (PECtwa, Time over Threshold) including different application pattern within two days using a random generator (application time slot 2 days from 8 a.m. to 6 p.m.). We calculated 25 different application scenarios. An adequate GIS-based software-environment and a functional workflow have been developed which make use of high and medium resolution geodata (water bodies, application areas, mitigating vegetation). The observed spatial entity here is a brook in the Hallertau Region, Germany. All hydrological parameters were derived from ground truthing data. Additionally a more generalised approach using artificial scenario-based landscape definitions will also be presented.
While the risk assessment assuming lentic water bodies is based on the comparison of the PECini with the RAC, the local exposure pattern predicted by this dynamic model is summarized to the maximum TWA (i.e. 1 h, PECTWA(1h)) and the total duration when the PEC is above the RAC (ToTh). Because the local PEC is depending on the variable timing and magnitude of the pesticide entries upstream, Monte-Carlo distributions provide a set of possible exposure patterns for each segment from which different PECTWA(1h) can be extracted (e.g. minimum, maximum, median). The exposure duration (as ToTh) is based on the calculation of a RACdyn to consider that effect thresholds are higher if the exposure duration is shorter.
The results show a continuous downstream increase of ToTh and a downstream increasing TWA strongly correlated to application patterns and the hydrological parameters. The artificial scenario-based results show thresholds of hydrological parameters of the flowing water systems where the influence of the hydrological conditions are the most important influencing parameters related to the simulated PECtwa.

EM02B-6

High resolution GIS maps for predicting the POPs contamination in soil and comparison with measured data

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The assessment of POPs contamination requires reliable spatial maps for burden and flux assessment. In this work, contamination maps were developed and validated at a space resolution of 1[GREEKX]1 m with a time frame of one day, in an experimental area located in the central Alps, where direct measurements of PCB concentrations in soil and environmental parameters were available for the year 2008.
Physical algorithms calibrated on experimental data were set up for temperature and organic carbon estimation, along the soil profile and across the year, in order to deduce the horizontal, vertical and seasonal distribution of the contamination potential for PCBs in soil (Ksa maps).
The developed maps were validated with an external set of PCB contamination data, giving very good results (e.g. for CB-153, R2 = 0.80, p-value ≤ 2.2·10-06). The obtained regression coefficients were used for the mapping of the actual soil contamination (concentration maps), taking into account the temporal shifts in soil concentrations from the equilibrium (as defined by Ksa values). These maps offer the opportunity to evaluate burden and fluxes with highly resolved temporal and spatial detail, and therefore with a high degree of ecological realism (emission maps).

EM02C-1

Comparison of far field and near field exposure from consumer products for chemical exposure prioritization

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Traditionnally fate and exposure modelling for Risk Assessment and Life Cycle Assessment of products has been focusing its effort on multimedia modeling of far field exposure. However, direct exposure of consumer products via either indoor air (for e.g. indoor furniture or flooring) or via direct dermal contact (e.g. for cosmetics or toys) may represent a dominant exposure. The present project therefore aims at developing metrics and method to consistently compare near field and far field exposure to chemical in products and at testing them on a serie of case studies of three consumer products.
Direct releases from consumer products are characterized by a new metric - the Product Intake Fraction - that determines the fraction of a chemical in a product that is taken up by humans during its use and disposal phase.
Measured releases for a pacifier plasticizer used in typical average conditions leads to relatively high product intake fraction of 6·10-2 and modeled product intake fraction for different shampoo ingredients are in the range of 10-3.to 10-1 depending on the considered chemical properties. For indoor releases of the flooring material, indoor intake fraction is high in the range of 10-4.to 10-2.
Comparison between impacts of direct consumer exposure to those linked to far field life cycle emissions shows that direct impacts are of the same magnitude as or higher than indirect life cycle impacts. Similarly the impacts of indoor releases of a flooring material are of the same order of magnitude as the respiratory effects of outdoor emissions. An uncertainty analysis shows that that the extrapolation from acute to chronic toxicity was the major source of uncertainty (54.1%) and that the three main substances contributing to the impacts also dominated the uncertainty with 84.0% of the total uncertainty.

EM02C-2

Assessment of local impacts of municipal solid waste management using life cycle assessment (LCA)

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This research takes part in a PhD work whose aim is to develop a methodology to assess locally environmental impacts of municipal waste management (MSW) in order to provide environmental elements to local decision makers and stakeholders.
MSW is a local issue managed under the responsibility of local authorities. To integrate environmental considerations, decision-makers often use Life Cycle Assessment (LCA). However, the LCA methodology does not take into account the characteristics of the territory involved and consequently not allow local assessment. Nevertheless, such a consideration appears necessary for local issues such as toxicity and odours. To solve the problem of assessing local impacts, the SETAC (Society of Environmental Toxicology and Chemistry) recommends the use of the Site dependent approach. This approach allows considering, in the characterization step, some spatial and temporal conditions of releases and some characteristics of the potentially affected environment in order to realize fate, exposure and effect analysis.
Our methodological research focuses on fate analysis step and its integration in the classification step. The novelty of our approach is the consideration of local environmental characteristics, through the USEtox model in a modified version, to locally assess toxicity and odours impacts. USEtox is a multimedia model which describes the fate, the exposition and the effect of substances released in the environment through three boxes (global, continental and urban). For spatial assessment of substances fate, we propose to change in the dimensions of the environmental boxes, compartments and in local relevant parameters which are determined by default in USEtox and not suitable for local assessment.
This work leads to the construction of a new set of spatial elements for classification and characterization steps for toxicity and odours impacts. The new classification step will be based on USEtox results from the new version and the consideration of spatiotemporal conditions of emission. The characterization step will be limited to the consideration of health effect and olfactory thresholds. The final objective is to compare classical LCA results (without taking into account spatial and temporal parameters) with located toxicity and odour LCA results. The spatial assessment of local impacts should allow policy makers and stakeholders to get "personalized"answers to debate within the decision-making.

EM02C-3

Coupled modelling of plant uptake, soil water balance and soil solute transport for estimating the fate of cadmium and lead in amended agrosystems

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Soil water balance, solute transport, leaching to groundwater and plant uptake of solute and water are coupled processes. Recently, a coupled plant and groundwater transport model for NaCl could simulate the transpiration-induced changes in groundwater salinity. However, for metals, no models that simultaneously predict plant uptake as well as leaching to groundwater were found. In this study, the water budget of soil, the uptake into plants and the leaching to groundwater of cadmium (Cd) and lead (Pb) were simulated simultaneously using a physiological plant uptake model and a tipping buckets water and solute transport model for soil.
Factors affecting uptake of trace metals into vegetation are type of metal, plant species and cultivar, plant-related parameters such as transpiration and growth, and soil parameters like pH, organic matter, soil texture and redox status. Robust tools for predicting the transfers of metals from soil and air to plants are scarce and often incorrect due to the large variability of metal uptake in plants.
The objective of this work is to present and test a model framework for the simulation of the coupled transport of water and dissolved trace metals, the uptake of both into crops, and leaching of solute and water to groundwater. The model is parameterized with data derived from a ten-year field study where four organic amendments were applied every two years.
Simulation results were tested on measured concentrations of lead (Pb) and cadmium (Cd) in soil and plants from the ten-year field study. Predicted concentrations slightly decreased with time in control soils, but increased in amended soils by about 10% (Cd) and 6% to 18% (Pb). Estimated plant uptake was lower in amended plots, due to an increase of Kd. Predicted concentrations in plants were close to measured levels in plant residues (straw), but higher than measured concentrations in grains. Initially, according to the model, lead was mainly deposited from air into plants (82% in 1998); later uptake from soil was dominating (30% from air in 2006), because of decreasing levels in air. For cadmium, uptake from air into plants was negligible (1-5%).

EM02C-4
Ranking of emissions of plant protection products from protected crops to environmental receptors
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A number of EU Member States requested EFSA to develop guidance on how to carry out environmental risk assessment of Plant Protection Products (PPPs) emitting from covered crops. In response, EFSA established a working group and commissioned supporting research to establish an inventory of protected crop systems and to determine the importance of emissions from these systems to relevant environmental compartments. The working group developed a classification system, consisting of 13 categories ranging from mulching and direct cover to high-tech greenhouses and closed buildings, in order to categorise the enormous variability in covered crop systems. The work on emissions performed so far revealed that emissions from covered crops may be substantial and might have been underestimated in the past. For the small and more open systems, it is expected that emissions do not substantially differ in comparison to open field systems. Comparison of emissions from selected closed walk-in structures with emissions from open field identified greenhouses and walk in tunnels having priority for scenario development for risk assessment. For these covered crop systems, the Panel on Plant Protection products and their Residues (PPR Panel) recommends the development of tiered risk assessment procedures. Further investigation is necessary to find out whether risk assessment for open field may be used as the first tier in these procedures. Furthermore, elements were identified that should be considered in deriving the risk assessment scenarios, for both soil-bound and soil-less cultivations.

EM02C-5
EU scenarios for exposure of soil organisms resulting from spray applications of pesticides to annual crops
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Based on a Member State consultation EFSA initiated a revision of the guidance for the soil exposure assessment including development of EU scenarios for numerical models. The aim of the exposure assessment was to obtain a 90th percentile of the PEC in space and time considering all fields in the regulatory zones North, Centre and South grown with the target crop where this active substance is applied. The types of ecotoxicologically relevant concentrations considered were both the concentration in total soil and the concentration in pore water averaged over the top 1, 2.5, 5 or 20 cm of soil (both peak and TWA concentrations for time windows up to 56 d). The scenario selection was based on a simple analytical model that calculates the concentration in soil assuming that degradation (first-order kinetics at a constant rate) is the only loss process from the 20-cm plough layer. The concentration in the pore water was calculated assuming a linear sorption isotherm assuming that sorption is proportional to organic matter. Concentration maps were calculated at a resolution of 1x1 km2 for the whole area of annual crops in each of the regulatory zones North, Centre and South. Simulations including uncertainty in the dry bulk density and in substance properties such as the Kom and the DegT50 showed that a spatial 95th percentile has to be selected for obtaining an overall 90th percentile when median or geomean values of these substance properties are used as input for the scenario calculations in the regulatory process. So the target was to select a spatial 95th percentile. Scenario were selected based on maps of the peak concentrations in total soil and in pore water that were generated for 19 substances and averaging depths of 1 and 20 cm. The selected scenarios for concentration in total soil have all relatively high organic matter contents and low temperatures. However, the selected scenarios for the pore water concentration have all relatively low organic matter contents because the pore water concentration is almost directly proportional to the inverse of the organic matter content for substances that show significant sorption. Calculations with the simple analytical model for a number of example substances showed that the concentration in total soil increased in the order South-Centre-North and that the concentration in the pore water increased in the order North-Centre-South (so opposite orders for these two types of concentration).

EM02C-6
Mechanisms behind conservatism in tiered soil risk assessment
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In recent EFSA opinions on assessment of exposure of organisms to substances in soil a rigorous scientific concept was presented to describe the spatio-temporal distribution of chemical substances in soil. Further ecologically relevant soil layers were derived. These layers represent the habitat of specific soil organisms used for risk assessment. For example the soil layer 0 1 cm is proposed for epigeic and anecic earthworms. The mean concentration for this layer (**E**cologically **R**elevant **C**oncentration) can be identified from the predicted concentration depth distribution. It is finally used for lower tier risk assessment (RA) in combination with an effect endpoint, e.g. a NOEC.
Though this approach appears straightforward it produces a surprising result. Compared to current soil RA it leads to dramatically increased ERC for a number of test organisms. On the other hand there are several publications comparing the current lower tier soil RA to higher tier (field) studies serving as reference tier. These came to the conclusion that the current soil RA is appropriate, i.e. it discriminates between critical and uncritical substances in sufficiently conservative way.
Why does the soil RA proposed by EFSA become so conservative and is this justified? A concept is proposed which analyses the whole process (design of lower tier effect study, derivation of endpoint, link to the exposure situation under real-world conditions) in order to provide answers to the above question.
The RA for earthworms is used as example because of the large number of studies available. Several factors may be considered to attribute to the different occurrence of effects in lower (lab) and reference tier (field) studies.
- Time to effect. Duration of study at reference tier typically 1 year, usually 56 days at lower tier (earthworm reproduction).
- Likeliness of proposed ecologically relevant soil layer, e.g. 0-1 cm. This habitat is very narrow. Extremes of soil moisture and temperature may render it temporarily to a hostile environment
- Impact of initial concentration. NOEC values usually in terms of initial concentration. Often experimental period at reference tier is long compared to DT50 of compound. Thus mean concentration is likely to be much lower than initial one.
These factors are investigated employing detailed analysis of the spatio-temporal concentration distribution in soil. Based on this information local effects can be estimated and aggregated on population level.

EM02D-1
Variability and estimation of pesticide half-lives in vegetation
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Half-lives in plants are among the most important drivers for the fate and behavior of pesticides in the environment, but also among the most uncertain parameters in multimedia models. We therefore aim at characterizing the variability of measured half-lives in plants collated from peer-reviewed literature and identify important predictor variables to provide an estimation model to predict bulk removal half-lives in various plants and plant components for a wide range pesticides. We analyzed more than 1000 data points covering a variety of reported half-lives in plants of almost 200 pesticides applied to at least one of 100+ plant species collected from 270+ peer-reviewed references published between 1956 and 2012.
Reported half-lives for synthetic pesticides range from 4.8 hours for dichlorvos sprayed on chrysanthemum flowers to 54 days for tebuconazole sprayed on wheat. The geometric mean half-life of all data points aggregated over pesticides and plant species is 4.1 days with a geometric standard deviation of 6.25. We identified a typical variability of less than a factor of 20 between reported half-lives. We also identified differences between removal from plant surfaces (usually leaves) and plant interior compartments, thereby giving insight into different contributing physical processes, such as photo-degradation exclusively important for plant surface removal. The number of data points per pesticide ranges from a single measurement for 28 pesticides to 55 analyzed samples for the well-studied insecticide endosulfan. We identified molecular weight, Kow, Koc, air and soil temperature, air and soil humidity, wind speed, surface-roughness, plant water and lipid content, soil pH and radiation intensity as potential predictor variables to be further analyzed for their influence on the degradation and other removal processes and for their reliability to be included into the final regression model.

EM02D-2
Predicting herbicide leaching to field drains in a clay dominated headwater catchment using MACRO
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The one-dimensional non-steady state MACRO model was used to help interpret observed propyzamide and carbetamide losses in an 8.6 ha underdrained headwater sub-catchment of the Upper Cherwell, with strongly undulating topography. Although the catchment is dominated by heavy clay soil, a small area of lighter and better drained soil over sandstone generates some groundwater contribution. Estimates of recharge to the sandstone aquifer were made using soil moisture balance calculations. A one-dimensional time-variant groundwater model was used to represent groundwater flow. Significant transfers of both herbicides to the drain network occurred soon after application. Peak concentration coincided with peak drain flow and concentrations then decreased gradually in a quasi-exponential fashion, mirroring the receding hydrograph. Observed carbetamide concentrations were about an order of magnitude higher that those observed for propyzamide due to a combination of a higher application rate and lower KOC. For propyzamide, total observed loss over the study period was estimated to be 1.1% of the applied mass and for carbetamide the loss was estimated to be 8.6%. MACRO predictions of the timing and magnitude of peaks in drainflow and herbicide concentrations were reasonable, particularly when corrected for baseflow contributions, although a period of a month when snow covered the ground led to a failure to represent peak flow. Hydrograph and chemograph recession was also reasonably well predicted. Nearly 100% of herbicide leaching to drains in this heavy clay soil is predicted to occur via macropore flow for both propyzamide and carbetamide. The results demonstrate the utility of one dimensional models as an explanatory framework for processes operating in larger undulating fields with significant topography. The results also confirm that drainflow is probably the dominant pathway for the transfer of these herbicides to the catchment outlet. This imposes considerable constraints on the management options available to reduce problematic herbicide exposure in this catchment.

EM02D-3
Nano-pesticides: state of knowledge, impacts on fate and exposure assessment
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Nano-formulations are already used in the pharmaceutical and food industries. In comparison, applications in the agrochemical sector are only emerging and a rapid growth is predicted in the upcoming years. Deliberate application of nanoparticles as within agricultural practises could be one of the rare intentional diffuse inputs of engineered nanoparticles into the environment (e.g. nano-silver). It is thus essential that risks and benefits to human and environmental health are adequately evaluated.
The aim of the present study is to (i) review the current state of knowledge on nano-pesticides, (ii) identify possible impacts on environmental fate and (iii) analyse the suitability of current pesticide exposure assessment procedures to account for novel properties within the EU regulatory context.
Nano-pesticides encompass a great variety of products and cannot be considered as a single category. The aims of nano-formulations are generally common to other pesticide formulations and consist in increasing the apparent solubility of poorly soluble active ingredient (a.i.), releasing the a.i. in a slow/targeted manner and/or protecting against premature degradation. Some nano-formulations are expected to have significant impacts on the fate of a.i. and/or to introduce new ingredients whose environmental fate is still poorly understood.
Current exposure modelling procedures are probably satisfactory for assessing the fate of most nano-formulations, provided that (i) realistic sorption and degradation parameters can be determined and used as model inputs, (ii) changes with time can be accounted for, if necessary, or (iii) the separate assessment of the various ingredients is proved to be a protective approach in all cases.
For formulations containing particles that may exhibit colloidal behaviour, further issues need to be considered since the transport of solutes and nano-particles cannot be described by the same approaches. The potential retarded/facilitated transport of the a.i. due to its association with a nano-carrier (e.g., polymer based or nano oxides associated with a.i.) should also be considered.
Further research is thus required in order to (i) identify the assumptions currently applied that are not valid in the case of nano-pesticides, (ii) evaluate the points or situations in which differences may impact significantly on the exposure assessment outcomes, and (iii) refine or adapt current protocols as required.

EM02D-4
On the effect of local pesticide reduction programmes in large river basins
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Large river basins have multiple sources of pesticides, which may be spread over more than 100-1000 km2. To effectively reduce the amount of pesticides entering surface water bodies, reduction programmes are implemented. The cumulative effect of pesticides entering the river system in upstream areas can compromise downstream water use e.g. raw water quality for drinking water abstractions. Local reduction programmes that target certain uses of pesticides may clearly affect local concentrations while the expected impact on the larger scale may fail to appear. For larger scale assessments pesticide fluxes coming from other sub basins and even transnational fluxes need to be taken into account. We illustrate the effect of a local reduction programme for the use of glyphosate on hard surfaces in the Meuse river basin. We used a long-term data set of glyphosate concentrations in the Meuse river to derive the trends in the concentrations before and after the implementation of the reduction programme. The results indicate spatial difference in the course of concentrations over time. In some areas of the river basin trend reversal is found, which might be possibly linked to programmes of measures but insufficient evidence is provided by the trend analysis to found this assumed action-effect relation.
Further modelling results complementing the monitoring data indicate spatial heterogeneity in the contribution of the sub basins in the targeted management area. The obtained regional insights are important for policy makers in prioritizing certain areas or approaches. In the Meuse river case, the hydrology of the catchment is such that the contribution from hard surfaces during summer is expected to have a larger impact on the downstream water quality because of low river discharges. Future recommendations are targeted monitoring in sub basins and at the outlets of waste water treatment plants and modelling the whole catchment to distinguish between sources and to derive cost-effective programme of measures.

EM02D-5
Application of a process based metamodel (MetaPEARL) to support vulnerability assessments of leaching to groundwater on different geospatial scales
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1. Introduction
A process based metamodel (MetaPEARL) is applied to support vulnerability assessments for leaching of pesticides to groundwater on different geospatial scales.
2. Materials and methods
The MetaPEARL model is based on a analytical model for pesticide leaching and a statistical evaluation of the simulations for 1000 scenarios and 56 pesticides with the numerical model EuroPEARL (Tiktak et al 2006). MetaPEARL requires a limited set of spatial data (precipitation, temperature, organic matter and texture) and takes into account the most important substance properties, available in registration dossiers (Kom and DT50).Geospatial information in form of homogeneous, EU-wide raster data are used in our exercise for MetaPEARL. The raster data, provided by the Joint Research Centre of the European Commission in cooperation with the European Food Safety Authority (EFSA), are the most recent and comprehensive information (http://eusoils.jrc.ec.europa.eu/library/Data/EFSA/). The maps contain information on soil, climate, land use, land cover and crops with a cell size of 1 km.
3. Results and discussion
We use the MetaPEARL model with the JRC/EFSA maps to determine the relative potential of pesticide leaching in the use areas of specific pesticides and crops in the EU. Taking into account the simplifications of the metamodel we provide relative vulnerabilities of scenarios to example pesticides rather than predicting concentrations.

Furthermore setting leaching studies into context of the area of use are explored by GIS analysis. The approach may support the evaluation of leaching studies generated under certain conditions in one region by setting them into context (on a relative scale) to the environmental conditions in other regions.

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EM02D-6

PEARL-BE: combining a meta-model and a proces-based pesticide leaching model to develop groundwater scenarios for Belgium

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Registration of pesticides on the European market requires a risk assessment of groundwater contamination following guidelines of the FOCUS workgroup. Nine worst-case standard FOCUS scenarios representing different soil types and climatic conditions are developed for Europe. In this study, the pertinence of standard FOCUS scenarios for Belgian conditions is evaluated and more specific scenarios are developed for each agricultural region using local information on soil and climate.

Local soil profiles were constructed using information from the Aardewerk-database (13 000 soil profile descriptions for Belgium) and the digital Belgian soil map. Within each agricultural region, soil profiles were parameterised using median soil properties for each soil type. Hydraulic soil properties were determined using pedotransferfunctions derived for Belgian soils. Daily meteo data for a 20-year period were available for 10 by 10 km grid cells. Crop calendars were parameterised according to the FOCUS Châteaudun scenario.

Modelling of pesticide leaching was done in a two-step approach. First Meta-PEARL was applied to all soil profiles to delineate vulnerable soils using median yearly net precipitation for each agricultural region. Soils with a predicted groundwater concentration close to the 80th percentile of PECs were considered as vulnerable soils. Next FOCUS PEARL was applied to the selected sensitive soils using daily meteo data for the centroid of each agricultural region. At the scale of Belgium, the predicted pesticide groundwater concentrations for 10 sensitive soils were compared to the results of 4 FOCUS scenarios (Châteaudun, Hamburg, Kremsmunster, Okehampton) to evaluate the pertinence of the FOCUS scenarios for Belgian conditions. Spring and autumn application of FOCUS substance B to sugar beet and winter cereal were compared. The Belgian scenarios were more critical than the FOCUS scenarios in all cases. At the scale of the agricultural region, FOCUS PEARL was applied to 3 sensitive soils and the scenario with the highest PEC was selected as the sensitive local scenario for the region.

The study shows that the results from the FOCUS scenarios are quite different from the results obtained with scenarios adapted to the local situation and based on more detailed information on soil characteristics. For Belgium, the use of local scenarios derived according to FOCUS guidelines is more conservative with respect to the protection of groundwater resources.

EM03 - Global scale modeling of environmental cause-effect chains for risk assessment and life cycle assessment: Quo vadis?

EM03-1

GIS-based regionalized Life Cycle Assessment: how big is small enough? Methodology and case study of electricity generation

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Few regionalized life cycle impact assessment method developers have justified their choice of spatial scale. In order for the promise of regionalized impact assessment to be realized, spatial scale should be chosen in a systematic fashion, and should match the spatial scale of the environmental phenomena that drive changes in characterization factors across space.

We propose the minimization of global spatial autocorrelation (AC) as a systematic technique to choose the spatial scale of impact assessment methods. Spatial autocorrelation is present when a data value can be inferred with minimal error from the data values of its spatial neighbors. The presence of AC can be interpreted as the presence of phenomena which are influencing CFs on a different spatial scale than the chosen one. The minimization of autocorrelation is therefore the choice of a spatial scale which best matches the spatial scale of the environmental or societal phenomena which are driving significant changes in CFs.

We calculated changes in global spatial autocorrelation at various spatial scales by using different techniques to aggregate disaggregated data. Our hypothesis is that the minimal spatial autocorrelation can be found by varying the number of spatial areas in the impact assessment method. In a case study for ecosystem damage due to freshwater consumption, global spatial autocorrelation was minimized at a spatial scale of 12.000 spatial units.

The choice of spatial scale is quite important in understanding, interpreting, and applying regionalized impact assessment methods correctly and with minimal uncertainty. Because the method proposed here makes no assumptions about spatial pattern or number of spatial areas, it can be applied to all regionalized impact assessment methods.

EM03-2

Spatially-explicit global scale modelling of acidifying air emissions

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Terrestrial acidification is the process in which atmospheric deposition, related to emissions of SO_2/SO_4, NO_x and NH_3, cause changes in soil acidity that can eventually harm terrestrial flora. Life cycle impact assessment (LCIA) uses characterization factors (CFs) to evaluate the consequences of terrestrial acidification. Current available LCIA methods modeling relies on regional to continental spatially-resolved models to evaluate atmospheric fate, soil sensitivity and the effect on the ecosystem, respectively. This represents a major limitation when assessing commodities produced and traded throughout a global economy. Consequently, this work shows a novel global scale approach to assess pollutant atmospheric transport and deposition, its consequences on soils, and its ultimate effect on plant species richness. CFs include an atmospheric fate factor (FF), a soil sensitivity factor (SF), and an effect factor (EF). FF describes the atmospheric impact pathway from the emission location to the corresponding deposition location, SF represents the soil pH change due to a marginal change in deposition and EF quantifies the biome-specific vegetation's "potentially not occurring fraction of species"(PNOF) due to a change in soil pH. FF derivation was based on the global tropospheric GEOS Chem chemical model. Changes in soil pH were evaluated with a geochemical steady-state model parameterized with a novel set of regional parameters at a global scale. EF was calculated from published regression and coefficients as a function of terrestrial biomes. CFs were then calculated through the sum of products of these three factors. CFs were obtained at a resolution of 2^ox2.5^o for SO_2/SO_4, NO_x, NH_3 and emissions. The obtained 2^ox2.5^o CFs results were also aggregated to coarser spatial resolutions: worldwide, continental and country levels. The outcome of this study is particularly useful for LCA practitioners who can now assess the consequences of a global economy while still maintaining the ability to differentiate emissions occurring between and within countries and thus enhance the environmental relevance of LCIA results. Furthermore, as worldwide spatial variability can now be assessed coherently, it gives the practitioners an idea of the uncertainty incurred from the choice of using coarser resolved (i.e. world, continental, country) CFs when the emission location is not "exactly"known.

EM03-3

Spatially-dependent freshwater concentration-species richness response functions for nitrogen and phosphorus

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Up to now, there are no concentration-species richness response functions for nitrogen (N) and phosphorus (P) in freshwater systems on a global scale. Reduced data availability and strong spatial specificity are reasons that challenge global scale modelling. The objective of our study is to (1) derive dose-response functions of aquatic species richness along both N and P concentration gradients for freshwater ecoregions worldwide and (2) compare the potential gain in precision and the increase in uncertainty by simulating different levels of spatial detail.

This was attained by gathering data from the literature (total of 472 studies) on the occurrence of aquatic species (diatoms, invertebrates, and bacteria, among others) along N and P concentration gradients. We gathered data on a total of 3707 species distributed across 110 freshwater ecoregions. In the presentation, we will (1) show results of the logistic regressions based on the empirical data we attained from the literature and (2) address the issues of increasing uncertainty and loss in precision by grouping ecoregions with similar characteristics with each other. This is a necessary step to assess the risk of nutrient pollution to world's regions for which very little data is available.

Our study tackles the issue of reduced data availability by performing a thorough empirical data gathering on a global scale. It also maintains spatial specificity and with the use of an ecoregion level of spatial resolution. Ultimately, our study provides a consistent method to compare the effects of N and P concentration to different aquatic organisms and different freshwater systems worldwide. Our results allow for ecologists to identify world's freshwaters that are most susceptible to nutrient concentration shifts. In addition, it is a valuable method to environmental risk and life cycle impact assessments since our results can be easily integrated to fate and exposure modelling so as to provide a cause-effect chain of freshwater eutrophication.

EM03-4

Including metal speciation in LCA terrestrial ecotoxicity: new regionalised characterization factors

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At present, ecotoxicological impacts of metals are often the main contributors to LCA results. This domination does not necessarily reflect a real toxicity but rather indicates the misuse of fate and effect models, initially developed for organic compounds and not accounting for metal speciation. A framework for including metal speciation in freshwater ecotoxicity in LCA has been developed recently. It relies on the use of a commercially available speciation model to define the bioavailable metal fraction and of freshwater archetypes to account for the influence of freshwater physicochemical properties on speciation. Although really interesting, this approach is not directly applicable to soils because speciation models were developed for aquatic environments. Soils greater heterogeneity hinders generalization and could invalidate hypothesis made in aquatic geochemical modelling.

The main goal of this project is thus to develop a method specific to soils, including defining the appropriate regionalization scale, in order to obtain terrestrial ecotoxicity CFs.

The Harmonized World Soil Database (HWSD) version 1.1 (FAO/IIASA/ISRIC/ISS-CAS/JRC, 2009) was used and enhanced to gather data on soil properties. The soil bioavailable metal fraction is computed with a soil specific parameterization of two available aquatic speciation models (WHAM 6.0 and MINEQL+), for each of the 5200 possible combination of soil properties of the world listed in the enhanced HWSD. The model parameterization is validated using field data. Multiple linear regressions are performed to detect the most influential soil properties on bioavailable metal fraction and soils are grouped in archetypes according to these properties. According to the framework proposed for freshwater ecotoxicity, a bioavailable factor is added to the definition of the CF and new effect factors are computed in terms of bioavailable fraction. Using the new regionalization scale here defined, new CFs are calculated for Zn, Ni and Cu for terrestrial ecotoxicity.

Results obtained for Zn indicate that it is possible to group soil types of the world in 70 different archetypes when considering the 9 most influential soil properties on speciation (R2=0,776). Model predictions are in good agreement with measured values (a difference of not more than one order of magnitude). Zinc bioavailability factors, with a variability of 20 orders of magnitude, show the importance of integrating speciation in LCA.

EM03-5

Evaluation of spatial variability of metal bioavailability in soils using geostatistics

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Soil properties show signifficant spatial variability at local, regional and continental scales. This is a challenge for life cycle impact assessment (LCIA) of metals, because fate, bioavailability and effect factors are controlled by environmental chemistry and can vary orders of magnitude for different soils. Here, variography is employed to analyse spatial variability of bioavailability factors (BFs) of metals at the global scale. First, published empirical regressions are employed to calculate BFs of metals for 7180 topsoil profiles. Next, geostatistical interpretation of calculated BFs is performed using ArcGIS Geostatistical Analyst. Results show that BFs of copper span a range of 6 orders of magnitude, and have signifficant spatial variability at local and continental scales. The model nugget variance is signifficantly higher than zero, suggesting the presence of spatial variability at lags smaller than those in the data set. Geostatistical analyses indicate however, that BFs exhibit no signifficant spatial correlation at a range beyond 3200 km. Because BF is spatially correlated, its values at unsampled locations can be predicted, as demonstrated using ordinary kriggin method. Similar approach can be employed for analyzing spatial variability of terrestrial ecotoxicity characterization factors of metals. Predicted maps can be used to provide a set of regionalized factors at spatial scales that are both scientifically relevant and practically feasible in LCIA.

EM03-6

A global assessment of the temporal and spatial variability of national dilution factors

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Of the many factors which influence the exposure of the freshwater aquatic environment to contaminating chemicals none has a more dramatic effect than dilution. Both where and when a chemical enters surface water will make an enormous difference to its impact on wildlife. This is of particular importance for down-the-drain"chemicals as these substances are discharged to freshwaters via sewer systems after consumer use. However, too often, this dilution capacity is fixed to a "generic"value. Although the spatial variability of dilution factors is often acknowledged, temporal variability is often unaccounted for which may potentially lead to underestimating the environmental risk. To address the magnitude of these dilution differences across the world, estimates of dilution factors were developed globally at a 0.5 degree resolution using gridded data. Thus, the focus here is on the numbers and location of the human population and the river water available to dilute their waste. The river flows estimates are calculated at both annual and monthly resolution based on readily available annual and monthly runoff estimates. The domestic waste water effluent is derived from combining gridded population and national per capita domestic water use estimates. For each grid cell both annual and monthly dilution factors were generated. This approach allowed the quantification of temporal and spatial variability of dilution factors not only at a catchment level but also at a national level, by means of statistical measures such as median and percentiles. This method revealed the dramatic differences in available dilution of chemicals both within and between countries , for example Canada has on average 4-orders of magnitude more dilution available than Tunisia, and Finland 3-orders of magnitude more than Spain. Over the course of a year, national dilution could vary between 10 and a 1000-fold depending on the country.

The work presented here is a significant step forward in terms of understanding the impact of river flow temporal variability on dilution factors at a national and global scale. The proposed methodology has great potential for scientists and decision makers across the globe, as it provides the means to improve screening-level chemical risk assessments.

EM0B1-6

Lead and lead isotopes in agricultural soils of Europe: natural distribution or contamination?

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In environmental sciences the 206Pb/207Pb isotope ratio is routinely used to argue for Pb contamination of different compartments of the environment. However, the lead-isotope background variation of a continent has never been established for any sample material. For the first time, a map of a Pb isotope landscape, based on samples of agricultural soils at the continental-scale, is presented. Agricultural soil samples (Ap-horizon, 0-20 cm), collected at an average density of 1 site/2500 km2 (2211 samples in total) from 33 European countries, were analysed for Pb concentration and Pb isotopes (206Pb, 207Pb, 208Pb). Lead concentrations vary from 1.6 to 1309 mg/kg, with a median of 16 mg/kg. Isotopic ratios of 206Pb/207Pb range from 1.116 to 1.727, with a median of 1.202.

The new data define the soil geochemical Pb background for European arable land, providing crucial information for geological, environmental and forensic sciences, public health, environmental policy and mineral exploration. The European continental-scale patterns of Pb concentrations and Pb isotopes show a high variability dominated by geology and influenced by climate. Lead concentration anomalies mark most of the known mineralised areas in Europe. At the continental scale of this survey, it is difficult to distinguish between natural anomalies, due to ore occurrences, and an amplified signal caused by mining and smelting. With the exception of some very local anomalies (related to cities, smelters), none of the observed patterns can be directly related to contamination, and all anomalies rapidly decrease to background values with distance to source. Combining the regional distribution of Pb concentrations with Pb isotopes, the conclusion is that the majority of lead in European agricultural land is still of natural origin.

EP01 - Antimicrobial resistance in the environment

EP01A-1

Characterization and comparison of multi-drug resistant methicillin-resistant Staphylococcus aureus [MRSA] from recreational beaches and high touch surfaces at a university and surrounding community

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Over the last decade community-acquired methicillin-resistant Staphylococcus aureus [MRSA] has emerged as a major cause of disease in the general population with no health care exposure or known classical risk factors for MRSA infections. MRSA colonization is a known risk factor for developing MRSA infections and MRSA are spread from fomite to person and from person to person. In a 2009 study we isolated and characterized 6 MRSA from public marine recreational beaches. The data suggested that there were multi-drug resistant MRSA in the beach environment and thus may be a reservoir for transmission of MRSA to beach visitors as well as a reservoir for macrolide and tetracycline resistance genes. The current study sampled and identified MRSA from local marine and fresh water recreational beaches which included sand, fresh and marine waters samples (n= n=296); from high touch surfaces on the University of Washington campus (n=294), in UW undergraduate housing (n=85), and the

local community (n=130). The presence of type I-VII of mobile Staphylococcal Cassette Chromosome [SCCmec], multilocus sequence typing [MLST] of the allelic profile of seven housekeeping genes, and the presence of aminoglycoside resistance gene, aadD; macrolide resistance genes, erm(A), erm(B) and erm(C), and msr(A); and tetracycline resistance genes, tet(M), tet(K), were determined by PCR assays and sequencing. Pulse field gel electrophoresis was done and the genetic relatedness of the isolates to USA300 was determined by Dice coefficient, UPGMA using the GelCompar II software. Strains that had > 80% homology with USA300 were classified as USA300. The highest level of MRSA positive samples [15.3%] were found in fresh water running into the marine beaches, where we frequently observed children playing during sampling, and at the fresh water beach on Lake Washington, while 11.7% of the surfaces from 5 of 8 undergraduate homes were MRSA positive. A surprising 98% of the 55 MRSA isolates were resistant to other classes of antibiotics and most likely represent reservoirs for these genes in the environment. In North America the majority of community acquired MRSA infections are due to USA300. This clone was found at the UW, in student housing and in the community but not in the recreational beach samples.

EP01A-2

Unique distribution of sulfonamide resistance genes, sul, in the Philippines aquatic environment

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EP01A-3

Tetracycline and mercury resistance genes in aquaculture sediments

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Farming fish in cages is an open system which has an effect on the chemical and ecological balance of the farming area. Fish feed, medicines and anti-foulants can affect microbial communities in the sediments under the cages. Considerable part of the substanced used ends up to the water outside the actual aquaculture facility either directly or via excrement of the fish. The aim of this study was to characterise the effects of aquaculture to the surrounding waters and sediments. In particular we were interested amount and diversity of tetracycline resistance genes (*tet*) and mercury resistance (*merA*) gene in comparison to pristine sediments. Four fish farms in the Finnish and Swedish archipelago were sampled during years 2006-2011.

Total DNA from top sediment samples was isolated with Fast DNA spin kit, Quantification of bioavailable tetracyclines and mercury was done by bioreporter bacteria. Total tetracyline content by was analysed by HPLC and total mercury by ICP-MS The resistance genes (*tetA, tetC, tetH, tetM* and *merA*) were quantified by qPCR.

No bioavailable tetracycline or mercury was found in any of the samples before 2011. LODs were 0,3 µg g^{-1} sediment for tetracycline and 0,04 ng g^{-1} sediment for mercury. The total amount of mercury, tetracycline and oxytetracycline was very low in all samples taken before 2011. However, there was high amount (more than 2 ng g^{-1}) of oxytetracycline present in one sample taken on 2011. The number of tetracycline resistance genes was clearly elevated in all fish farm samples until 2009 and the number of mercury resistance genes was also slightly elevated. Samples taken after that are currently under analysis. Diversity of *tetC* gene was non existent and diversity of *tetM* was relatively low. In contrast, the diversity of *merA* was considerable. The aquaculture farms had used tetracyclines but ceased that about a decade ago so the reasons for the elevated tetracycline resistance genes is not obvious, like are the reasons for accumulation of *merA* gene. Very low diversity of tetracycline resistance genes may implicate a common source for the genes. The *merA* sequence diversity in both fish farm samples was similar, phylogenetic analyses showed that there was a little difference between sequences from different sampling sites.

EP01A-4

Abundance of streptomycin and tetracycline resistance genes in apple orchards treated with streptomycin in comparison to untreated apple orchards

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Streptomycin is the only antibiotic authorized for use in plant agriculture within the EU and Switzerland. Its use is authorized on an annual basis for the prophylactic treatment of apple and pear orchards against the bacterial disease fire blight. Tetracycline is currently the only other viable alternative should resistance to streptomycin emerge in the fire blight pathogen; Erwinia amylovora. We have developed a multiplex qRT-PCR for the relative quantification of streptomycin and tetracycline resistance genes, with the 16S rRNA genes as the endogenous control. Using these multiplex qRT-PCRs we have monitored the abundance of streptomycin and tetracycline resistance genes in streptomycin treated and untreated orchards in 2010 and 2011.

2. Materials and methods

Sample of flowers, leaves and soil were collected from three orchard sites at each time-point (265 samples per year). The time-points consisted of prior to streptomycin spraying, one day after the streptomycin spraying, two weeks after streptomycin spraying and at apple harvest. The DNA extraction method and the relative abundance of resistance genes were detected as previously described.

3. Results and discussion

The abundances of strA and strB genes increased in the flower and leaf samples over time in comparison to the untreated samples in 2010 and 2011. However, the harvest samples contained a similar abundance of strA and strB genes to the samples prior to streptomycin spraying. There were no streptomycin influenced changes in the abundance of streptomycin resistance genes in the soil samples. The relative abundances of the tetracycline resistance genes tetB, tetM and tetW were not affected by the treatment with streptomycin in the flower, leaf or soil samples. There were low fluctuations within the abundances of the streptomycin and tetracycline resistance genes within the samples isolated from the untreated orchards over time.

4. Conclusions

There were short term increases in the abundances of strA and strB genes associated with streptomycin treatment in the flower and leaf samples. However, the abundance of these resistance genes returned to pre-treatment level at harvest. Streptomycin treatment did not influence the abundance of streptomycin resistance genes in the soil samples nor did it influence the abundance of tetracycline resistance genes within the orchard samples. Thus, the streptomycin associated increases in resistance gene abundances are temporary.

EP01A-5

Antimicrobial resistance and their selectors in pig manure and sewage sludge

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Antimicrobial resistance is a pestering problem, and the solution still remains to be found. Due to a supposed reservoir function of the environment, environmental contamination with antibiotics and / or resistant bacteria is a serious issue. In addition, bacteria are hard to tracew, once spread into water or soil. Therefore, it is the more necessary to monitor contaminated effluents before they enter the environment.

We investigated the antimicrobial resistance of bacteria - comprising *E. coli*, enterococci, lactobacilli and clostridia - which were isolated from sewage sludge and pig manure. The statistical association of antimicrobial resistance with different potentially selective factors was assessed in a linear model.

Chemical precipitation in sewage plants was significantly associated with increased bacterial resistance in sewage sludge. Antibiotic contamination and heavy metal contents were significantly associated with bacterial resistance in pig manure. *E. coli* from manure samples with supermedian contents of copper (> 11.8 mg / kg manure wetweight) were significantly more often resistant against ampicillin and piperacillin. *E. coli* from manure samples with zinc contents > 22.75 mg / kg manure wetweight were significantly more often resistant against ampicillin, doxycycline and piperacillin.

In order to track whether environmental isolates might be transferred to humans, we investigated by ERIC-PCR the genetic relatedness of *E. coli* from pig manure, sewage plants, and human hospitals. In general, different phylogenetic groups prevailed among isolates from pig manure and human hospitals; isolates from sewage sludge resembled partly isolates from pig manure, partly isolates of human hospitals. Two closely (or clonally) related *E. coli* isolates were found in a sample of pig manure and a human stationary patient. Interestingly, despite the close phylogenetic relatedness, both isolates carried different resistance genes. On the contrary, identical resistance gene profiles were present in phylogenetically unrelated isolates from humans and pigs. These findings indicate a major role of horizontal spread in the distribution of antimicrobial resistance among *E. coli*.

EP01A-6

Occurrence and dissemination of antibiotic resistance genes in anthropic environments

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Dissemination of antibiotic resistance genes is recognized to occur in various environmental compartments although it remains difficult to demonstrate in complex environmental matrices. Some environments have been defined as putative hot spots for gene transfer as they can sustain high microbial cell densities and combine both antibiotic and antibiotic resistance bacteria. In this study we compare the occurrence of class 1, 2 and 3 integrons (mobile genetic elements mobilizing different resistance cassettes according to their respective class) from wastewater, sludges, farm slurry and manure. Same or equivalent environmental matrices were also evaluated for their propensity to support the transfer of a model integron-bearing plasmid (pB10).

In farm samples, only class 1 and class 2 integrons could be detected. Prevalence of integrons was significantly more important in the farmyard manure than in the slurry. In

WWTP influents, the occurence of class 1 integrons was significantly more important than the other classes. We could show that WWTPs as a process reduced the relative concentrations of integrons by 2 log. In dynamic point of view, plasmid pB10 did not appear to disseminate in farm manure microcosms while maintaining steadily over the course of the experiment. In activated sludge microcosms, pB10 did not persist because of an apparent loss of the donor bacteria. Nevertheless, the dissemination of the plasmid appeared as an increasing plasmid to donor ratio in microcosms setup with sludge from anaerobic digesters or fixed biofilm reactors
Regarding integrons, the difference of occurence may reflect more or less homogenous anthropic selective pressure on antibiotic resistances. On the other hand, propensity for pB10 to transfer sludge microcosms seems to rely on the origin of the sludge ecosystem from a given treatment process.

EP01B-1
Detecting evolutionary hot spots of antibiotic resistances in Europe (DARE)
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Antibiotic resistances (ARs) are a great threat to human health, which has meant that research in this area has focused primarily on their role within clinical settings. This is in strong contrast to the research in nonclinical environments, such as the natural and urban environment, where this research has only recently received more attention. Here the first results of a European wide network COST Action are presented. The Action consists of 84 members of 18 European Nations and Israel which try to identify the gaps of knowledge, which need to be investigated in order to propose interventions to curb the spread of AR evolution and AR microbes within the environment. The Action has developed criteria for antibiotics and bacterial organisms and environmental hot spots of AR evolution, which should be investigated as first priority. Furthermore, in order to assess the risk of antibiotic resistance evolution a new approach of risk assessment is needed. However, the Action has also identified here the largest gap of knowledge. Currently, no systems exist which will allow to assess the risk of spread or evolution of new antibiotic resistances. Therefore investigations to fill this gap of knowledge are strongly called for and this is also the case for research resulting in a first geographic pattern on AR resistance in Europe. Such an epidemiological base information exists already for clinical and veterinary data, but is missing for environmental data.

EP01B-2
Selective pressure of antibiotic pollution on bacteria of importance to public health
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Many bacteria of clinical importance survive and under certain conditions may grow in different environments. Antibiotic pollution may exert on them a selective pressure leading to an increase in the prevalence of resistance. The objective of this study is to determine whether measured environmental concentrations (MEC) of antibiotics and concentrations used as action limits in environmental risk assessment (ERA) may exert a selective pressure on bacteria of importance to public health in the environment. Species sensitivity distributions were derived for ciprofloxacin, erythromycin and tetracycline using endpoints calculated from minimum inhibitory concentration distributions of clinically relevant bacteria. The potentially affected fraction (PAF) of bacterial taxa at MEC of antibiotics and ERA action limits was used as a proxy for antibiotic selective pressure. MEC and ERA action limits were also directly compared to wild-type cut-off values. The PAF of bacterial genera at antibiotic concentrations measured in water environments is estimated not to exceed 6%. MEC in river sediments, swine feces lagoons, liquid manure and farmed soil are estimated to inhibit wild-type populations in up to 49%, 84%, 100% and 23% of bacterial genera, respectively. At concentrations used as action limits in ERA, erythromycin and ciprofloxacin are estimated to inhibit wild-type populations in up to 15% and 65% of bacterial genera. Concentrations of antibiotics measured in different environments and used as action limits in ERA are high enough to exert a selective pressure on clinically relevant bacteria that may lead to an increase in the prevalence of resistance.

EP01B-3
Monitoring and management of antibiotic resistance of veterinary drugs in Germany
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The use of antibiotic drugs in veterinary therapy results in a target conflict of demand for therapy vs. antimicrobial resistance. Advantages such as secure animal health of companion and farm animals, avoidance of economical loses in animal husbandry and prevention of bacterial zoonoses are opposed by the risk of the enhancing antimicrobial resistance in humans and animals. A management approach is the responsible use of antimicrobials applying the "ONE HEALTH Principle" (animals + humans = one health) of the WHO and EU (animal health strategy 2007-2013). This strategy needs information on the current status of antimicrobial resistance. This information is obtained from monitoring programs conducted and improved in Germany for more than 10 years.
These include:
National resistance monitoring - animal pathogens
National food monitoring program
Monitoring of resistance for zoonosis germs
National Monitoring of resistance for commensal germs
Additional information is obtained from the collection of postmarketing data (ADRs, PSURs etc.) and since 2011 from data of antibiotic disposal to be registered by law.
This talk presents some of these data and shows management actions drawn from these data and put into force both nationally and within the whole EU.

EP01B-4
Reduction of pathogens and antibiotic resistance genes by Soil Aquifer Treatment (SAT) in the Mézquital Valley, Mexico
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Aquifer recharge presents advantages for integrated water management, namely, treatment of reclaimed water and additional dilution of pollutants due to mixing with natural groundwater. Nevertheless, this practice can represent a health and environmental hazard because of the presence of pharmaceuticals, pathogens and antibiotic resistance genes. The Mézquital Valley, 60 km north of Mexico City, is the world's largest wastewater (WW) irrigation area. The WW from Mexico City is transported without treatment to the Mézquital. There, approximately 800-900 km2 of agricultural land are irrigated with this untreated WW. We followed the WW on its way to the Mézquital taking samples from seven sites starting with the effluent of a big hospital in Mexico City and ending with water from a spring that arises after SAT in the Mézquital. Total DNA was extracted from the water and applied to quantitative real-time PCR (qPCR) targeted to the 16S rRNA gene of bacteria, the 23S rRNA gene of Enterococcus and to six resistance genes mediating resistance to fluoroquinolones, sulphonamides and C-lactam antibiotics. Total bacterial load was reduced by almost three logs from 1.75 [GREEKX] 109 16 S rRNA gene copies / 100 ml WW to a final concentration of 3.73 [GREEKX] 106 16 S rRNA gene copies / 100 ml spring water destined for drinking water purification. A four log reduction to 1.66 [GREEKX] 103 copies / 100 ml water was detected for the nosocomial pathogen, Enterococcus. For the resistance genes, the highest concentration was found for the sulphonamide resistance gene sul1 with a concentration of 1.14 [GREEKX] 109 gene copies / 100 ml WW, which decreased to 1.38 x 104 copies in the spring water. The fluoroquinolone resistance genes qnrA and qnrB were present in the WW at 5 x 105 copies / 100 ml; both were completely eliminated on their way to the spring. In summary, two of the six resistance genes were completely eliminated, the others were reduced by three to five logs from the WW to the spring. Mixing of WW with rainwater, passage through a dam, and sedimentation of particles suspended in WW hardly affected bacterial load and resistance gene abundance. The major cleaning effect was exerted by SAT in the Mézquital. Natural WW treatment, like sedimentation, mixing with rainwater and SAT, can considerably decrease biological contaminants in water. However, total bacteria, nosocomial pathogens and resistance genes were not satisfactorily removed to use this water without further treatment as drinking water.

EP01B-5
Impact of treated wastewater irrigation on antibiotic resistance in agricultural soils
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Antibiotic resistance (AR) is a global phenomenon with severe epidemiological ramifications. It is becoming increasingly clear that anthropogenically-impacted natural aquatic and terrestrial "hotspots"may be playing a significant role in the global proliferation of AR because these environments serve as gene reservoirs which can be transferred to clinically relevant (potentially pathogenic) organisms via water and food webs. Treated-wastewater (TWW) irrigation is becoming increasingly prevalent in arid regions of the world, due to growing demand and decline in freshwater supplies. The release of residual antibiotic compounds, antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs) from wastewater effluent may result in proliferation of AR in irrigated soil microcosms. The aim of this study was to assess the impact of TWW-irrigation on soil ARB and ARG reservoirs. Tetracycline, erythromycin, sulfonamide and ciprofloxacin resistance was assessed in agricultural soils irrigated in tandem with either freshwater (FW) or TWW, using standard culture-based isolation methods and culture-independent molecular analysis using quantitative real-time PCR (QPCR). Significant

levels of native bacterial antibiotic resistance were detected in both FW- and TWW-irrigated soils. Nonetheless, ARB and ARG levels in TWW-irrigated soils were on the whole identical (or sometimes even lower) than the FW-irrigated soils, despite detection of relatively high levels of both ARB and ARG in the TWW. This indicates that the high numbers of resistant bacteria that enter the soils from the TWW are not able to compete or survive in the soil environment; and suggests that the impact of the TWW-associated bacteria on the soil microbiome is on the whole negligible. We therefore conclude that AR in the soil is primarily associated with native resistance of the indigenous soil microbiome and not with introduced strains or genetic elements.

EP01B-6

Urban wastewater disinfection and solar irradiation of wastewater polluted stream: effect on antibiotic photo-degradation, inactivation of antibiotic resistant E. coli strains and their resistance

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Two wastewater disinfection processes (UV radiation and chlorination) were tested in the inactivation of two previously selected E. coli strains and their resistance to three antibiotics (Amoxicillin (AMX), ciprofloxacin (CPX), and sulfamethoxazole (SMZ)). Next, disposal of urban wastewater treatment plant (UWWTP) effluent into surface water was simulated and the effect of solar irradiation on the photodegradation of target antibiotics, the inactivation of antibiotic resistance E. coli strains and their resistance were investigated. Two different strains of E. coli resistant to the target antibiotics (higher antibiotic resistance, HAR; lower antibiotic resistance, LAR) were isolated from wastewater sample and subsequently inoculated in previously autoclaved wastewater samples. Total inactivation by UV radiation (250 W wide spectrum UV lamp) was observed after 60 min in LAR E. coli strain inoculated wastewater (4.0 106 UFC/100 mL) but not after chlorination process. Additionally, unlike of chlorination process, UV radiation effected resistance of LAR E. coli strain to CPX (MIC decreased from 12 to 8 µg/L). When simulated solar irradiation effect was investigated on wastewater polluted surface water, E. coli strains inactivation was found to be really low (26 and 50% respectively). According to wastewater disinfection tests, solar irradiation effected resistance of LAR strain to CPX too, but MIC was decreased to 8 µg/L only after 180 min irradiation. Finally, the effect of solar irradiation on antibiotics (1 mg/L initial concentration respectively) photodegradation rate resulted in the following order (half-life time): CPX (t1/2= 24 min) < AMX (t1/2= 99 min) < SMX (t1/2= 577 min). In conclusion, antibiotics and ARB may be released into the stream from UWWTP effluents partially unaffected, and because of the lower efficiency of solar irradiation, the risk of horizontal transfer and selection of antimicrobial resistance genes among bacteria can increase.

EP02 - Endocrine disrupting chemicals: recent developments

EP02A-1

Zebrafish eleutheroembryos provides a suitable vertebrate model for screening thyroid gland disrupting chemicals

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Thyroxine-immunofluorescence quantitative disruption test (TIQDT) was designed to provide a simple, rapid, alternative bioassay for assessing the potential of chemical pollutants and drugs to disrupt thyroid gland function. This study demonstrated that zebrafish eleutheroembryos provided a suitable vertebrate model, not only for screening the potential thyroid disrupting effect of molecules, but also for estimating the potential hazards associated with exposure to chemicals directly impairing thyroxine (T4) synthesis. Amitrole, potassium perchlorate, potassium thiocyanate, methimazole (MMI), phloroglucinol, 6-propyl-2-thiouracil, ethylenethiourea, benzophenone-2, resorcinol, pyrazole, sulfamethoxazole, sodium bromide, mancozeb, and genistein were classified as thyroid gland function disruptors. Concordance between TIQDT on zebrafish and mammalian published data was very high for those chemicals with a direct effect on the thyroid gland function, as the sodium-iodide symporter (NIS) and the thyroid peroxidase (TPO) inhibitors. The physiological relevance of T4-intrafollicular content was clearly higher than regulation at the transcriptional level of tg or slc5a5. Moreover, concentration-response analysis provided information about the thyroid disrupting potency and hazard of selected positive compounds. Finally, the effect of perchlorate, a NIS-inhibitor, but not MMI, a TPO- inhibitor, was completely rescued by low-micromolar amounts of iodide. TIQDT performed on zebrafish eleutheroembryos is an alternative whole-organism screening assay that provides relevant information for environmental and human risk assessments.

EP02A-2

All mixed up: phenotypic plasticity in a genotypic world

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The processes of sexual differentiation and development are dependent upon an intricate cascade of molecular signals and deviations from normal can have catastrophic effects on individual fertility and fitness. Previously, several studies have shown that exposure of *Xenopus laevis* to sufficiently potent estrogens at critical times during development results in feminization and/or demasculinization, including male to female phenotypic sex reversal at adequate doses. However, given that genotyping of *X. laevis* has only recently become possible, studies performed in the past were rarely able to make concrete linkages between genetic and phenotypic sex. Therefore, to further characterize the relationship between genotype and phenotype, *X. laevis* tadpoles were exposed to 0.1, 1, or 10 µg/L 17α-ethynylestradiol (EE2), the estrogen analog commonly used in oral contraceptives, from 12 h post-oviposition through 13 wks post-hatch. All EE2 treatments resulted in significant delays in time to metamorphosis. Genotyping showed that genetic sex ratios were similar among treatments. However, morphological evaluation revealed that phenotypic sex ratios were altered in all EE2 treatments. Interestingly, complete male to female phenotypic sex reversal was rare at the concentrations tested, and a surprising number of individuals displayed intersex gonads, abnormal gonads, and atypical vitellogenin over-expression, that were only diagnosed upon histological examination. The impacts of these conditions on fertility and fitness are not known but are likely to be adverse and more complex than complete sex reversal. In all likelihood, the relatively great number of intersex and abnormal animals is a result of estrogens functioning downstream of the initial molecular signals of sexual differentiation. Thus, genetically male animals receive mixed endogenous male and exogenous female signals that cause disordered sexual development. The vitellogenin over-expression was probably temporally independent from primary effects on sexual differentiation and likely drove the significant delays to metamorphosis that were observed in all EE2 treatments. Expression analysis of mRNA, as determined by *Illumina* transcriptome sequencing, is ongoing to help characterize the transcriptional signature of abnormal sexual development and to allow further assemblage of the molecular pathway that leads to the disordered development.

EP02A-3

Androgen-induced kidney hypertrophy in the European bullhead (Cottus sp.): a potential biomarker of androgen exposure

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The present work was designed to identify an androgeno-regulated signal in the kidney of the European bullhead (Cottus sp.) and to characterise response patterns prior to laboratory and field applications to address androgenic potential of chemicals. In a first step, gender dependent ultrastructural modifications of kidney were quantified using the Kidney Epithelium Height (KEH) measurement. During breeding period, male bullheads exhibited an increased KEH value of 20.96 ± 3.57 µm compared to female (16.96 ± 1.28 µm). No gender difference was recorded in non-breeding bullheads. In a second step, bullheads out of breeding period were exposed 14 or 21 days to different concentrations of androgens (0.5 or 5 µg/L of trenbolone (Tb), 1 or 10 µg/L of 11-ketotestosterone (11KT), 0.5 or 5 µg/L of Spironolactone (SPI), 0.5, 5 or 50 µg/L of Dihydrotestosterone (DHT)), estrogen (0.05 or 0.5 µg/L of Ethinyl-estradiol (EE2)) or heavy metal (1 or 10 µg/L of Cadmium (Cd)). Results showed that androgens are able to induce kidney hypertrophy. Trenbolone induced a dose-dependent hypertrophy (KEH= 16.59 ± 0.81 µm at 0.5 µg/L and 21.01 ± 1.76 µm at 5 µg/L vs 15.58 ± 0.85 µm for the controls) as the higher concentration of 11-KT (KEH = 17.55 ± 1.48 µm at 10 µg/L) and DHT (17.43 ± 1.77 µm at 50 µg/L). SPI and Cd have no effect on kidney ultrastructure. In bullheads exposed to EE2, a trend to decrease of KEH was observed. In a next step, wild bullheads were electrofished in 9 streams located in the North of France and characterised by various contamination levels. KEH responses don't allow to discriminate both reference (KEH = 16.03.± 1.25 µm) and contaminated (KEH = 16.17 ± 1.08 µm) sites. This work highlights an androgen-regulated signal in the kidney of the European bullhead. However, kidney hypertrophy appears as few sensitive and time consuming indicators. Also, identification of associated protein and/or RNA modifications could provide a relevant biomarker of androgen exposure.
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EP02A-4

Endocrine disrupting compounds as potential obesogens: musk compounds as a case study

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Since the discovery of leptin and other adipokines it is now clear that the adipose tissue is not only a storage place for excessive fat but a real endocrine organ, making it possibly sensitive to endocrine disrupting compounds (EDCs). Recently 'the environmental obesogen hypothesis' stated that environmental pollutants such as EDCs could

play a role during the development of metabolic diseases such as obesity, broadening the endocrine disrupting concept to a more physiological disruption concept including endocrine, neural and metabolic disruption. This hypothesis could, together with an increased caloric intake, a sedentary lifestyle and genetic predisposition, give potential complementary explanation to the recent epidemic proportions of obese and diabetic patients.
Musk compounds are a group of synthetic EDCs used in a variety of personal care products and therefore often daily used. Synthetic musks enter the systemic circulation mainly trough dermal absorption and have been detected in human adipose tissue due to their high lipophilicity (Log kow = ± 5). During this study we evaluated the potential obesogenic properties of three musk compounds belonging to the different classes of musks: the nitromusk Musk xylene; the polycyclic musk Tonalide® and the macrocyclic musk Ethylene Brassylate.
The adipogenic potential of the test compounds were evaluated using the 3T3-L1 cell line, a model in vitro cell system for the study of adipogenesis. These cells are fibroblastic and can differentiate into adipocytes after a ten-day exposure with an adipogenic cocktail (isobutylmetylxanthin, dexamethasone and insulin). The Adipored assay, a fluorescent staining for the quantification of lipid droplets associated with the phenotype of mature adipocytes, was used to screen the three musk compounds for their adipogenic potential. Tonalide® was the only compound inducing a dose dependent increase of the lipid droplet formation. The effect of Tonalide® on the differentiation of adipocytes was further confirmed by measuring the gene expression of an adipocyte specific marker gene adipocyte specific protein 2 (aP2) during exposure.
The Adipored assay as well as the expression of the adipocyt specific marker gene aP2 show that Tonalide® is the only musk compound, from the three tested, inducing the differentiation of adipocytes in vitro. In the next step, microarray analysis and RNAi technology will be applied to unravel the adipogenic mechanisms of Tonalide®.

EP02A-5
Development of gene expression biomarkers in cetaceans skin biopsies exposed to bisphenol A (BPA) and perfluorooctanoic acid (PFOA): new tools for emerging contaminants assessment
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Currently, one of the main toxicological issues, in the management and conservation of the marine environment, is the study of the potential impact of compounds released from plastics, such as bisphenol A (BPA) and industrial derivates, such as perfluorooctanoic acid (PFOA). On this regard, the assessment of toxicological risk in wildlife requires the development of sensitive biomarkers including those based on the use of in vitro systems. BPA is one of the most distributed compounds in the world, both in the aquatic and terrestrial ecosystems acting as agonist or antagonist for endocrine receptors. The perfluorinated compounds are used as surfactant and in the surface treatments, they are persistent and it has been shown that they can act as endocrine disruptors as well. To develop new gene expression biomarkers in cetaceans we exposed skin biopsies of three odontocetes species (sperm whale, killer whale, and bottlenose dolphin) to BPA and PFOA. We selected two potential biomarker genes such as the peroxisome proliferator-activated receptors α and γ (PPAR α and γ). The PPARs belong to a superfamily of ligand-dependent nuclear receptor (PPARα, β and γ) which regulates physiogical processes of lipids homeostasis, inflammation, adipogenesis, reproduction, etc. PPARα and PPARγ seem to be modulated by the presence of BPA and PFOA respectively. The two genes of interest (PPARα, PPARγ) were sequenced in three odontocetes species. The mRNA levels were quantified in response to the two different treatments in the slice samples. Four genes (PPARα, PPARγ, and the previously developed Erα and E2F1) are modulated by the treatments in all the three species. In particular, the results of this set of experiments, revealed that the BPA treatments induce the expression of the genes PPARα and PPARγ showing a dose-response trend. Increasing the BPA concentration increases the bottlenose dolphin, killer whale and sperm whale slices mRNA levels, as well as for E2F1 apart from the killer whale. On the opposite, the PFOA exposure shows a down-regulation of the PPARα and PPARγ both in sperm whale and killer whale slices, while ERα and E2F1 are poorly induced by PFOA in both species. These data represent the first evidence of emerging contaminants effect on cetaceans based on an in vitro experiment and suggest the potential use of this diagnostic markers as early warning signal of exposure to plastic released compounds and emerging contaminants in marine wildlife monitoring.

EP02A-6
Detection of estrogenic and androgenic chemicals in waste waters and peri-urban water bodies in Bulawayo, Zimbabwe
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Endocrine disrupting chemicals (EDCs) as environmental contaminants have received considerable attention since their discovery. In this study, the yeast estrogen and androgen screen tests were adopted to detect the presence and potencies of estrogenic and androgenic endocrine disruptors in treated effluents from sewage treatment plants (STPs), Thorngrove (THORN) and Aisleby (AIS) STPs, an urban river (Matsheumhlope River), and effluent receiving peri-urban dams (Umguza Dam and Khami Dam) in the industrialised and semi-arid City of Bulawayo, Zimbabwe. Pollutants in water samples were extracted by C18 Solid phase extraction (SPE) . Estrogenic activities of extracts were expressed as 17β-estradiol equivalence quantities (EEQs). Estrogenic activity was detected in the effluents of both STPs with EEQ values of 32.8 ng/L and 55.3 ng/L for THORN and AIS STP respectively. In Umguza Dam water, estrogenic activity was extremely high, showing the highest EEQ of 236.5 ng/L while Khami Dam had EEQ of 8.5 ng/L. Matsheumhlope River, an urban river, had the least EEQ value of 2.2 ng/L. Androgenic activity was only detected in AIS STP, with dihydrotestosterone (DHT) equivalence quantity (DHTEQ) of 93.1 ng/L. The results indicate that the STP effluents have high concentrations of estrogenic chemicals and urban/ peri-urban dams, the ultimate sink of all waste waters from the city, are laden with estrogens with potential to cause reproductive disorders in fish and other aquatic organisms. The study can also indicate the potential adverse effects of inadequate waste management practices in developing countries; which are rarely given much attention in urban xeno-biotic pollution studies. We recommend 'follow-up' field based studies to determine the effects of these xeno-estrogens on reproduction of fish and other aquatic organisms in the peri-urban water bodies.

EP02B-1
Assessment of exposure to estrogenic contaminants in bile extracts of red mullet from Western Mediterranean: an integrated chemical and biological approach using the ER-LUC assay.
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In this work we have identified and measured the concentrations of major metabolites of polycyclic aromatic hydrocarbons (PAHs) and alkylphenols in field collected bile extracts of red mullet (Mullus barbatus) from Mediterranean Spanish waters. Hydroxylated-PAHs (1-naphthol, 9-phenantrol, 9-fluorenol, 1-pyrenol, 1OH-BaP and 3OH-BaP) and alkylphenols levels (4-n-nonylphenol (NP) and 4-tert-octylphenol (OP)) were quantified in fish bile samples by gas chromatography-mass spectrometry in electron ionization mode (GC-EI-MS). In addition we have applied the estrogen responsive luciferase (ER-LUC) reporter gene assay to measure total estrogenic activity in the same bile extracts of male fish. By integrating the results of both analytical chemical and bio-analytical approaches, we have attempted to explain the measured ER-LUC activity by the calculated potencies based on chemical analysis of hydroxylated PAH and AP metabolites. Our results showed consistent spatial differences in concentrations of hydroxylated-PAHs (ΣOH-PAHs), alkylphenols and estrogenic activity in bile samples, which are presented and discussed.

EP02B-2
Mixtures of the most commmon antiandrogenic pesticides act additively in vitro: implications for risk assessment
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Evidence suggests that there is a widespread decline in male reproductive health and that mixtures of pesticides may be an important causal factor. At present, 1252 active plant protection products are registered in the US and 411 in Europe, with another 72 "pending". With such a high number of products on the market, it would be practically impossible to test enough combinations to make robust conclusions about their potential to impact the environment and humans. Therefore, the accurate predictability of mixture effects using modelling approaches is essential for risk assessment. We tested mixtures of pesticides with androgen receptor (AR) antagonist properties using a fixed-mixture ratio design. The selection criteria for inclusion in the mixture was based on estimated exposure (high), antiandrogenic activity (active), registration status (current) and toxicity (less than 10 µM) and led us to select 13 pesticides. We used the MDA-kb2 assay to test 3 mixtures: 1. Pure antagonists (8mix: fludioxonil, fenhexamid, ortho-phenylphenol, tebuconazole, dimethomorph, methiocarb, pirimiphos-methyl, p,p'-DDE); 2. Dual function agonists/antagonists (5mix: cyprodinil, pyrimethanil, vinclozolin, chlorpropham, linuron); 3. All (13mix). Concentration addition (CA) and independant action (IA) models were calculated to predict mixture responses. All the mixtures were within the "prediction window"of CA and IA, whereby CA tended to overestimate, and IA to underestimate, effect. The 8mix agreed with CA, but not IA, whereas the 5mix and 13mix agreed with either CA or IA, or both, depending on inhibition level (10% or 50%) and mixture ratio. For the first time, this shows that multi-component mixtures of widely used pesticides can act together in a predictable and additive manner. Significantly, the mixture tested here is composed of some of the most widely used pesticides found on foods in Europe and the USA, mosttly fungicides, indicating that the potential for exposure to mixtures of these test pesticides is high. Considering that risk assessment procedure does not incorporate mixture scenarios at present, it is possible that risk to human health and environment is being underestimated, and therefore, effects of mixtures should be taken into account.
*Orton, F., E. Rosivatz, M. Scholze, and A. Kortenkamp, 2011.Widely used pesticides with previously unknown endocrine activity revealed as in vitro anti-androgens. Environ Health Perspect, 119: p. 794-800.

EP02B-3
Endocrine and anti-androgenic potency of by-products in flame retardants
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Some brominated flame retardants (BFRs) have unintended negative effects on the environment and human health (e.g. endocrine effects). Some of them show a strong bioaccumulation in aquatic and terrestrial food chains, some are very persistent, and some show serious toxicological effects such as endocrine disruption [1-5]. During the last decade an increasing number of reports have presented evidence of these negative effects caused by some BFRs. Less toxic alternatives appear to be available already but comprehensive information on their possible toxicological effects and exposure are lacking. The European Commission-funded project ENFIRO investigates halogen-free substitution options for some BFRs resulting in a comprehensive dataset on viability of production and application, environmental safety, risk assessment, and life cycle assessment. In total 15 halogen-free flame retardants (HFFRs), consisting of metal-, organic-, and nano-based FRs, as alternatives for decaBDE, TBBP-A, and brominated polystyrenes were selected. These HFFRs were assessed in a toxicity screening to provide information on the hazards. This paper presents the results of the hazard characterisation and shows that impurities in some technical HFFR products were responsible for in vitro estrogenic and anti-androgenic activity. The identity of the impurties that were responsible for the endocrine effects could be resolved with LC-HRTOF-MS and NMR.

EP02B-4

Investigation of endocrine disruption in Australian aquatic environments - stage 1

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This project uses an integrated approach consisting of multiple in vitro and in vivo bioassays, in situ sampling and trace chemical analysis to compare endocrine disruption at 73 sites in mainland Australia. Sample sites were selected to include waterways impacted by a variety of sources such as wastewater discharge, agricultural run-off, industrial effluent, urban drains and pristine reference sites. Duplicate 1 L discrete water samples were taken quarterly over a one-year period. Samples were concentrated using solid-phase extraction (SPE) and split into two aliquots, one for in vitro bioassay analysis and the other for chemical analysis. A battery of CALUX assays (estrogen receptor, ER; androgen receptor, AR; progesterone receptor, PR; and glucocorticoid receptor, GR) was used to determine the classes of EDCs present in water extracts. Chemical analysis was used to identify causative compounds as well as for point source confirmation. Preliminary results have identified at least 11 sites that have estrogenic (or anti-androgenic) EDCs. Analysis with the AR-, PR-, and GR-CALUX assays is currently underway. Chemical analysis has confirmed estrogen mimics (such as bisphenol A, t-octylphenol), the pesticide atrazine, an industrial compound tris(2-carboxyethyl)phosphine (TCEP), and numerous pharmaceuticals and personal care products (e.g., atenolol, dilantin, triclosan, carbamazepine, ibuprofen). Hormone analysis is currently underway. Based on in vitro and chemical data, several stock solutions representative of a selection of exposure concentrations found in the environment will be created for in vivo laboratory fish exposures using a native species (rainbowfish, Melanotaenia fluviatilis) and a widespread exotic pest species (mosquitofish, Gambusia holbrooki) in stage 2. An estrogenic biomarker (vitellogenin) and an androgenic biomarker (in development) will be used to assess whole organism endocrine disruption. The same endpoints will be utilized with in situ techniques by sampling fish from polluted aquatic environments identified in stage 1 to assess the amount of endocrine disruption present in the most impacted natural aquatic environments. Finally, in stage 3, a risk assessment will be generated using in vitro, chemical, in vivo, and in situ data to assess the potential risk to aquatic ecosystem health.

EP02B-5

Applying tissue-burden based quality benchmarks to assess the ecological risks of endocrine disrupting organotin compounds in Hong Kong waters

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Since their application as biocides in the 1960s, organotins (OTs), in particular tributyltin (TBT) and triphenyltin (TPT), have caused widespread adverse effects on marine organisms such as the induction of imposex (i.e., superimposition of male sexual characteristics - penis and vas deferens - on females) in over 200 species of neogastropods, and growth inhibition and deformities in oysters. A mandatory global ban on the use of OT-based antifouling systems has been enacted since September 2008. It is, therefore, anticipated that there will be a reduction of OT pollution in marine environments around the world. In this study, we measured the imposex status and tissue concentrations of OTs (i.e., mono-BT, di-BT and TBT, mono-PT, di-PT and TPT) in the rock shell *Thais clavigera* collected from 28 coastal sites of Hong Kong during summer 2010. The results indicated that *T. clavigera* from a number of sites contained high TPT concentrations and suffered from high degree of imposex. The average TPT tissue concentration was 11,108 μg kg^{-1} dry weight (dw) in *T. clavigera* collected from Aberdeen, which was 26 times higher than the maximum TBT tissue concentration in the animals obtained from Kadoorie Beach (i.e., 422 μg kg^{-1} dw). Using the Monte Carlo approach, an ecological risk assessment was conducted by computing the distribution of risk quotients (RQs); RQ is the ratio between a measured tissue concentration of the target pollutant (MTC) and predicted effects tissue concentration (PETC) (i.e., RQ = MTC/PETC). The results showed that 11.1% of *T. clavigera* across all sites in Hong Kong waters was at risk with RQ > 1 due to exposure to TPT, whereas the risk associated with TBT was relatively low (0.7% with RQ > 1). Kadoorie Beach, Butterfly Beach, Waterfall Bay and Aberdeen, the four sites which are close to the shipping facilities, were severely impacted by TBT, while all 28 sites were alarmingly heavily contaminated by TPT. As TPT is highly toxic and may trigger imposex in *T. clavigera*, the local rock shells are still under considerable threat associated with this pollutant even after the global ban of OTs in antifouling systems. This study highlights that TPT is a major environmental concern which deserves immediate actions to control its use and release, and to remediate its pollution in the marine environment of Hong Kong and the Pearl River Estuary.

EP02B-6

Multi-parameter assessment of endocrine disruption in Irish marine waters using biological effects measurements, chemical analysis and passive sampling

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An integrated study examining the uptake and concentrations of selected endocrine disrupting compounds (EDCs) in transplanted Mytilus edulis was carried out at 3 sites on the coast of Ireland. The study combined the biological effects alkali-labile phosphate assay (ALP) and the estrogen luciferase reporter assay (ER LUC); chemical analysis using liquid chromatography tandem mass spectrometry (LCMSMS), and the Polar Organic Chemical Integrative Sampler (POCIS). The exposure was conducted over a three month period with mussels transplanted from a reference site to cages at three coastal locations; Dublin Bay and Galway Bay impacted by secondary treated wastewater effluent and a reference site with little to no anthropogenic input in Galway County on the Irish West coast. Results from the exposure study showed that levels of selected EDCs and their resultant effects in the Irish marine and estuarine waters studied were low. Mussels and water samples were analysed for the estrogenic EDCs estrone, 17β estradiol, 17α ethynyl estradiol, nonylphenol, octylphenol and bisphenol A using liquid chromatography/ tandem mass spectrometry (LC-MS/MS). A similar ratio of the steroid estrogens estrone and 17β estradiol was detected in water samples and POCIS. The levels detected were in the low ng/L range for water samples, and ng/device for POCIS. The highest concentrations of steroid estrogens were detected in POCIS in July at all sites, with lowest levels observed in September. EDC levels in mussel tissue were detected at low ng/g concentrations. ALP and ER LUC results varied between sites. The natural cycle of seasonal ALP and ER LUC responses in the transplanted mussel stock was used to normalise levels detected in the mussels after transplantation and exposure. Results from this integrated study provide a comprehensive assessment of the levels and potential for biological effects of selected EDCs in Irish marine waters. The suitability of transplanted mussels as biomarkers in environmental monitoring programs is discussed. Transplantation of bio-monitor species, the collection of multi-parameter biological effects and analytical data in addition to collection of data from 'less impacted' areas, when corrected for natural processes, will be vital for the derivation of appropriate future assessment criteria and may be suitable to further support legislative objectives under Marine Strategy Framework and Water Framework Directive monitoring.

EP02C-1

Reproductive responses of two native freshwater fish species populations of Chile, after 5 years of bleached kraft mill effluent discharge and discharge diverting to sea

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Between 2007 and 2011, we monitored the reproductive status of wild populations of native fish in a basin of central Chile, the potential effect of exposure to effluent discharges from ECF pulp plant cellulose with a modern production and processing system (greenfield mill). We have confirmed endocrine disruption at different levels of biological organization in populations of two species of native freshwater fishes of Chile (*Percilia gillissi* and *Trichomycterus areolatus*) downstream the discharge during periods of 2007 to 2009. Depending on the seasonality of monitoring have shown an induction or inhibition of the production of sex steroids, gonadal maturation and histological level increased or decreased during periods of recrudescence and spawning. In January 2010, the plant began to discharge into the sea, but due to the earthquake

of February 27, 2010, the plant discharged into the river again briefly. Despite there was a high variability in the size of the gonad of *P. gillissi*, we observed a decrease in gonad size, along with induction of EROD activity downstream of the discharge. *T. areolatus* showed no differences with the reference sites for the same period. During periods of 2010 and 2011, there have been recoveries of populations downstream of the discharge, especially in *T. areolatus*. While there is a difference in the sensitivity and resilience of the species, is showing greater resilience in T. areolatus. Both species appear to exhibit an overstimulation of the reproductive system during exposure to effluents from pulp, which carries a positive and/or negative feedback of the reproductive system, dependent on the gonadal maturation period to be monitored. These changes have not reached a critical threshold of disturbance, as there is evidence of resilience in both species, by suspending the discharge of effluents from pulp to its habitat.

EP02C-2

Polycyclic aromatic hydrocarbons and endocrine disruption: the role of junctional intercellular communication

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Male reproductive function in animals and humans is considered to be a system highly sensitive to many chemicals and physical agents generated by industrial or agricultural activities. Recently, many disturbing trends have been observed in male fertility, such as decreasing sperm counts, deteriorating semen quality and increasing frequencies of malformations of testis and incidence of testicular cancer. Endocrine-disrupting Chemicals (EDCs) are discussed as possible cause of these adverse trends in male reproductive health. In addition, to estrogen- and androgen-mediated processes, there is a strong evidence that testicular cell-to-cell communication mediated by gap junctions, termed Gap Junctional Intercellular Communication (GJIC), is involved in testicular development, regulation of hormone release, cell differentiation, initiation, and maintenance of spermatogenesis. Thus, inhibition of GJIC during critical stages of development may result in male reproductive dysfunction leading to infertility. Indeed, many chemicals known to be EDCs modulate GJIC and/or impair connexin expression in gonadal or non-gonadal cells. However, there is limited information on the detailed role of GJIC in adverse reproductive effects caused by specific EDCs. Recent studies indicate that anthropogenic air pollutants can possibly impair reproduction of human and wildlife. It has been reported that Polycyclic Aromatic Hydrocarbons (PAHs) on airborne particulate particles compromised sperm functions and altered endocrine hormone levels in exposed animals. Our study addressed the endocrine-disrupting potential of air pollution as a source of compounds that may alter male fertility. The inhibition of GJIC by PAHs and air samples was assessed in testicular cells in this experiment, in order to determine whether PAHs could cause endocrine disruptive effects by closing gap junction channels. This research was supported by GACR P503/10/P249, the SoMoPro project number 2SGA2764 (funded from the European Community within the Seventh Framework Programme (FP/2007-2013) under Grant Agreement no. 229603 and co-financed by the South Moravian Region) and by the Brno PhD Talent Financial Aid from Statutory city of Brno to Petra Kubincova.

EP02C-3

Improving the in vivo predictive value of in vitro estrogenicity bioassays by combining in vitro results with kinetic characteristics of estrogenic compounds

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Stably transfected reporter gene In vitro bioassays such as the ER-Luc bioassay (T47D or U2OS cell-based) or the transcriptional activation assay (using HeLa-9903 cells) provide rapid and sensitive, effect-directed tools for quantifying the estrogenic potency of compounds and sample extracts. A limitation when using these in vitro bioassays for in vivo hazard assessment, however, is that the ADME (absorption, distribution, metabolism and excretion) characteristics of compounds are not taken into account, which can hamper the in vivo predictive value of these bioassays. Our aim was to alleviate this limitation by taking specific ADME characteristics into account.
Our present work compares the in vitro estrogenicity of a suite of selected (xeno-)estrogenic compounds relative to the reference compound ethinylestradiol (EE2) and literature-derived in vivo estrogenic responses obtained with the uteroptrophic assay using subcutaneously exposed rats. The selection of compounds includes alkylphenols, benzophenone derivates, isoflavones, phenyl methanes and steroids. A methodology was applied that calculates EE2 equivalencies by not only taking the in vitro derived estrogenic potency of these compounds into account, but also compound-specific, in vitro differences in hepatic availability (the dose escaping hepatic clearance) relative to EE2 as determined by incubations with rat liver microsomes and cytosol. In addition, compound-specific differences in serum protein binding were assessed using equilibrium dialysis in order to determine the available concentrations of the selected compounds.
The studies demonstrate that combining in vitro estrogenic potency with in vitro determined compound-specific kinetic characteristics for hepatic clearance and protein binding does improve the correlation with the in vivo effect doses obtained with the rat uterotrophic assay, and quantifies the impact of these factors. The improved in vivo predictive value of the in vitro estrogenicity bioassays contributes to the derivation of an in vitro effect-based benchmark dose needed for risk assessment of e.g. drinking water samples containing estrogenic compounds. The approach also offers future perspectives for other in vivo predictions based on in vitro determined toxic potencies.

EP02C-4

Quantifying thyroid hormones and steroids in wastewater effluents by using a Mode of Action-based tool-box

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Study of Endocrine Disrupting Chemicals has focused largely on estrogenic compounds. However, it has not been until recently when researchers expanded the scope of their work to xenobiotics that may perturb thyroid hormone homeostasis and/or interact with nuclear steroid receptors for steroids (i.e. estrogens, androgen, progesterone, and glucocorticoid receptors). Simultaneously to the advances in the Environmental Sciences (chemistry, and (eco)-toxicology) and Engineering to address the risks associated with the presence of EDCs in the environment, there has been an increased public awareness and demand of information by citizens and water utilities.
Whole Effluent Toxicity (WET) is a permit requirement in the U.S. that EPA uses to implement the Clean Water Act's prohibition of the discharge of toxic pollutants in toxic amounts. WET refers to the aggregate toxic effect to aquatic organisms from all pollutants contained in a facility's wastewater (effluent). WET tests measure wastewater's effects on specific test organisms' ability to survive, grow and reproduce. In anticipation of WET requirements of wastewater facilities to monitor the increasing number of EDCs in wastewater and meet the pertinent regulations, a comprehensive set of tools needs to be implemented. A Mode Of Action-based approach, in which a battery of bioassays and chemical analysis are combined to identify toxic effects and their causing agents, is proposed for this purpose.
The current work encompasses a multi-laboratory and multi-disciplinary effort to monitor and characterize the endocrine disrupting activity of effluents from wastewater treatment plants, and the specific chemicals that may underlie these effects. The primary endpoints targeted in this project are thyroid disruption and estrogenic and progestagenic effects.
In this presentation, the results from monitoring selected steroids (estrogens, androgens, and progestins) and thyroid hormones, as part of the initial chemical characterization of a set wastewater effluents, will be shown. Additionally, the equivalent disrupting potency calculated from the measured concentrations by chemical analysis will be compared to the results from a battery of ex vivo and in vitro bioassays (i.e. thyroid gland explant culture assay, thyroid peroxidase inhibition assay, rat and human pregnane-X and progesterone cell-based nuclear receptor reporter assays, and vitellogenin gene expression assay with larvae Fathead Minnows).

EP02C-5

Statistical analysis of histopathology endpoints

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Histopathology experiments, especially in the aquatic environment, are usually conducted with multiple subjects (e.g., fish) in each of multiple vessels (e.g., tanks) within a water control and each of several doses or concentrations of the test material. Responses are typically ordered severity scores on each subject with values 0 for no effect, 1 for minimal effect, through 4 or 5 for severe effect. The usual analysis for such responses from a 1-generation study is to do separate Mann-Whitney or Dunn's nonparametric comparisons of treatment groups to control, where the data are either replicate means or medians, or else replicates are ignored and all subjects are analyzed as independent observations. The mean score is statistically unsound and both it and the median score ignore much important information. Furthermore, these tests on median scores make no allowance for the common phenomenon of varying numbers of subjects in different vessels. On the other hand, ignoring replicates and treating all subjects as independent ignores the experimental design and frequently observed phenomenon that subjects in the same vessel tend to have responses correlated differently from subjects in different vessels. These pairwise approaches also ignore the biological expectation that severity of effect tends to increase with increasing concentrations. A step-down Jonckheere-Terpstra test, applied either to replicate medians or ignoring replicates, uses the presumed monotone concentration-response but otherwise suffers from the same limitations as pairwise methods.
A new test, the Rao-Scott Cochran-Armitage by Slices, or RSCABS, is proposed that incorporates the replicate vessel experimental design and the biological expectation that severity of effect tends to increase with increasing doses or concentrations, while retaining the individual subject scores and revealing the severity of any effect found. A statistical protocol is proposed for analyzing both 1-generation and multi-generation histopathology studies. It is illustrated with data from three recent multi-generation medaka studies done by a joint Japan-USA project under the auspices of the OECD Validation Management Group for Ecotoxicity.

EP03A-2
Stability of functionalized gold nanoparticles in water systems with different composition
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EP03A-3
Application of field-flow-fractionation for the analysis of engineered nanoparticles in complex matrices
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EP03A-4
Quantitative analysis of fullerenes in soil samples
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Fullerenes are carbon-based nanomaterials present in the environment due to several natural events including volcanic activity, meteoritic impacts, lightnings and wildfires, and due to the human activity, including combustion processes, car-brakes, etc. During the recent years a new source of fullerenes to the environment can be expected through the increasing development of nanotechnology. Thus, a general consensus exists in considering fullerenes as a new family of emerging pollutants.
Recent studies have identified the presence of C60 and C70 fullerenes in atmospheric particulate [1] and wastewater samples [2]. Nevertheless, analytical methods for these compounds are somehow scarce, especially in the case of solid matrixes. Traditional extraction techniques have been applied for this purpose with limited success [3] and laser-desorption ionization sources have been extensively applied for their mass spectrometric analysis despite little quantitative information can be achieved and they entail risk of fullerene self-generation. There is a need of developing reliable, robust, accurate quantitative methods which allow to assess the occurrence of fullerens in soil and sediment matrices.
In the present work, ultrasound assisted extraction and accelerated solvent extraction techniques have been explored and both methods have been optimized and validated for the analysis of eight functionalized and unfunctionalized fullerenes in soils. The method allows the detection and quantification of fullerenes in the pg/g order. In order to assess the presence of fullerenes in real soil matrices, 58 real soils have been analyzed. The results and their relation to fuel-burning/industrial/urban activity will be further discussed.

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EP03A-5
Sedimentation of nanomaterials in natural waters
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There is a growing need for risk assessment of different nanomaterials in order to support their safe production and use. For this we need to estimate the exposure concentration of nanomaterials in the environment. We focus on estimating the exposure concentration in water based on removal process such as sedimentation and dissolution. In this study we measured the sedimentation and dissolution of different nanoparticles, PVP capped Ag, Silica coated Ag, CeO2 and C60. In order to cover a broad range of particle properties metal, metaloxide and carbon particles were used. Due to use of HR-ICPMS relatively low concentrations of metal particles could be tested. These particle related properties are combined with the environmental properties of 6 different water types which range in ionic strength, dissolved organic carbon, acidity and suspended solids. Additionally the effect of the suspended solids is taken into account by comparing sedimentation in filtered and unfiltered water samples. Sedimentation rates and residual concentrations are derived from the observed data. The dissolution measurements show that removal of nanoparticles from the water phase due to dissolution is neglegable, except in sea water where up to 10% of added Ag particles dissolved. The removal due to sedimentation ranges from less than 8% to almost 100%. This depends greatly on water characteristics, but large difference are also found between particle types, e.g. PVP vs Silica coated Ag particles. In general high dissolved organic carbon concentration increased stabilization against sedimentation. The description of sedimentation by first order removal kinetics towards a residual concentration seems valid. This is valuable for predicting the exposure concentration of nanoparticles in the aquatic environment.

EP03A-6
River biofilms and silver nanoparticles: de novo nanoparticle formation and nanoparticle weathering
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This work focusses on the effects of the extracellular matrix of river biofilms on silver nanoparticles (Ag NPs) and introduces new possibilities to visualize nanoparticles in environmental samples.
River biofilms are the main producers of biomass and oxygen in freshwater ecosystems. Ag NPs will first interact with the extracellular matrix (EM) of periphytic organism before the latter are actually exposed. EM components may change the properties of the nanoparticles and thus influence the interaction with and effects on the organisms. EM is extracted from biofilms colonized in indoor channels in a flow-through system fed with river water and is analyzed for cell lysis, protein content, TOC/DOC content, DOC size distribution.
Ag NPs and AgNO3 are diluted in NaHCO3 with 10mg/L EM in the dark or the light. The samples are characterized for the presence of NPs and the size (dynamic light scattering (DLS), NP tracking analysis (NTA), electron microscopy (EM)), stability (electrophoretic mobility), Plasmon resonance (UV-VIS), dissolution (ICP-MS), and chemical composition (ESRF).
AgNPs: Dispersions remained stable in NaHCO3 at pH 6- 8.6 over one week in both light and dark. The addition of EM lead to a slight size increase but dispersions remained stable in the dark. Exposure of Ag NPs to EM and light lead to pH-dependent weathering over time.
AgNO3: There was no significant nanoparticle formation in the dark in the absence of EM and a low rate of particle formation in the light. In the presence of EM and light, de novo particle formation was observed within minutes and at a slower rate in the dark. Particle size was pH dependent. The presence of a Plasmon resonance signal shows that at least parts of the NPs are elemental Ag.
We are currently measuring the chemical composition of the weathered and newly formed particles.
In situ characterization: With a new approach we use CLSM and NTA to characterize NPs in biofilms. The size of Ag NPs was thus determined in intact biofilm samples. We are now going to track particle size and formation in biofilms over time. The chemical identity of the NPs in the biofilms will be analysed via CLSM-Raman Spectroscopy.
Ag NPs, Ag+, and EM components from river biofilms form a dynamic system which is influenced by pH and light. Our results indicate that wherever there is Ag+ and EM in the environment, nanoparticles may form. This is essential information for the assessment of the potential environmental effects of AgNPs.

EP03B-2
Respiratory distress and biochemical changes in brain but no impact on behaviours of rainbow trout exposed to nanoparticulate titanium dioxide
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Since the advent of nano-ecotoxicology a number of studies, including several from our research group, have demonstrated very subtle biochemical changes in brains of fish exposed to engineered nanoparticles (NPs). This has led us to investigate the implications of these effects for fish behaviour; behaviours link physiological and ecological processes and are key determinants of fish population structure. In this study investigating the effects of TiO_2 NPs on locomotor and social behaviour of fish, juvenile rainbow trout (23.7 ± 3.7 g) were exposed to 1 mg/L TiO_2 NPs (24.5 ± 10.6 nm, primary particle size) or an equivalent concentration of TiO_2 bulk material (134.1 ± 42.5 nm). Spontaneous fish movements were quantified with Ethovision video tracking software (Noldus Information Technology) and outcome of interactions between fish scored by direct observation; juvenile rainbow trout are aggressive and outcome of paired interactions between trout from different treatment groups may reveal fitness disparities. Following 14 days exposure fish from both treatment groups exhibited morphological changes to gill tissue, most notably a characteristic swelling of primary and secondary lamellae, compared to control fish. However, elevated concentration of red blood cells and haemoglobin were only observed in fish exposed to TiO_2 NPs, suggestive of a nano- specific effect. Biochemical changes including elevated (1.5 fold) lipid peroxidation were also observed in the brain of fish in fish exposed to TiO_2 NPs. Element analysis revealed accumulation of Ti at gill of fish but no significant accumulation was observed in internal organs, including brain, suggesting toxicity observed was driven

by interaction of TiO_2 NPs at the gill and TiO_2 NPs are not bioavailable. Whilst these data indicate that exposure to TiO_2 NPs may cause biochemical disturbance in rainbow trout, locomotion was unaffected. Video tracking of movements revealed no significant difference in distance travelled control (195 ± 37 m/h, mean ± SEM, n = 5), bulk TiO_2 (167 ± 31 m/h, n = 5), or TiO_2 NPs (117 ± 26 m/h, n = 6) exposed fish. Outcome of paired interaction was also not affected. These data suggest TiO_2 NPs cause toxicity in rainbow trout but respiratory distress and brain injury exhibited by fish in this study does not impair ecologically relevant behaviours.

EP03B-3

The Four S's of Nanoparticle-Cell Interactions: size, shape, surface chemistry and serum proteins

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There are currently over 1000 consumer products on the market that contain or utilize nanomaterials, and this number is expected to increase exponentially in the near future in the absence of detailed knowledge concerning the interactions of such materials with biological systems. While nanoparticles have been shown to cross cell membranes, little research has examined the influence of particle characteristics on membrane transport. Furthermore, our inadequate understanding of potential human and ecological effects has resulted in uncertainties of the potential risks of this technology, and impeded the advancement of quantitative risk assessments. The goal this project was to characterize the influence of particle size, shape, and surface chemistry on the movement of gold nanoparticles across mammalian cell membranes, as well as the influence of serum protein concentrations on this uptake. A549 carcinomic human alveolar cells were utilized to characterize the movements of gold nanoparticles across cell membranes. Specifically, the absorption of three different gold nanoparticle shapes was examined at multiple sizes: spheres (5, 20, and 50 nm), cubes (50 and 75 nm) and rods (20x100 and 20x200 nm). The influence of surface chemistry on absorption was examined through surface modifications of these particles of various shapes and sizes by polyethyleneimine, polyethylene glycol, and citrate. Each particle was characterized by TEM, DLS, UV-vis, and zeta potential. In total, comparisons of cell uptake were examined for 21 different modifications of gold nanomaterials. Also, the influence of serum protein concentrations on this uptake was investigated for nanospheres with anionic and cationic surface modifications. Cells were plated in 12-well plates at 100,000 cells per well and exposed to 1 mg/L gold for 0.5, 1, 1.5, and 2 hours. Bioaccumulation of nanoparticles at each time point was quantified by inductively coupled plasma-mass spectrometry (ICP-MS), and movement was visualized via confocal microscopy. This research provides a foundation upon which to predict biological interactions with nanoparticles and facilitate future risk assessment endeavours.

EP03B-4

Trophic transfer of gold nanoparticles in a water food chain

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The risk of nanoparticles is becoming an issue. Nanoparticles are ultrafine particles and can be easily penetrated through cell membrane. To understand their potential risk, we investigated trophic transfer of nanoparticles in a water food chain. The low and high tropic level organisms of this study were green algae *Chalmydomonas reinhardtii* and invertebrate *Daphnia magna*. *Chalmydomonas reinhardtii* was initially exposed to gold nanoparticles (AuNPs; 10 nm), and 2-day old *Daphnia magna* were fed by the exposed cells (2.5 [GREEKX] 10^5cell/ml). The results showed that gold nanoparticles can transfer from *Chalmydomonas reinhardtii* to *Daphnia magna* via food exposure. This study demonstrated that nanoparticles can be transferred from the algae to invertebrates in aquatic ecosystem. *This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2011- 0015985).*

EP03B-5

The chronic toxicity of ZnO nanoparticles in Daphnia magna and the application of different detection methods to assess their bioavailability

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Zinc oxide nanoparticles are being increasingly produced and will therefore somehow end up in the aquatic environment. Although many different acute toxicity studies have been performed, the number of chronic toxicity data is still very limited. However, when looking at environmental relevant scenarios the knowledge on chronic toxicity is of great importance. Additionally, there are still many conflicting data on whether the toxicity caused by ZnO nanoparticles is due to the ion fraction or to the nanoparticle itself. Therefore, we investigated the chronic toxicity of ZnO nanoparticles in a well characterised model species *Daphnia magna*. During 21 days the daphnids were exposed to a concentration range of ZnO nanoparticles (Alfa Aesar). Different endpoints such as reproduction, mortality and growth were checked. In order to determine the speciation of the nanoparticles the concentrations of total zinc, ZnO nanoparticle and zinc ion were measured in the daphnia test medium. Unfiltered and filtered (0.45 µm filters) medium samples were taken at different times of exposure and the zinc concentration was analysed by ICP-OES. Another technique called Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) was used to measure free Zn^{2+} concentrations. In order to distinguish between the ion, nano and aggregated fraction, ultrafiltration via different filters was used. The different fractions were analysed via ICP-MS. The results of the chronic exposure experiment revealed an EC_{50} value around 0.02 mg/L for the reproduction of *D. magna*. The growth and mortality of the daphnids were significantly influenced at the highest exposure concentrations. The results of the first filtration technique indicate that there were no significant differences in zinc concentrations between the unfiltered and filtered (0.45 µm) medium samples directly after spiking (0 h). In contrast, after 48 h, the zinc concentration decreased significantly in the filtered samples compared to the unfiltered ones. This indicates a certain aggregation of the particles in the first 48 h after spiking. Preliminary results for the AGNES technique show that only about half of the zinc oxide nanoparticles dissolved into zinc ions. Further research is needed to confirm the chronic toxicity results and to elucidate the link with the bioavailability of the ZnO nanoparticles under different forms.

EP03B-6

Effect of non-ageing and ageing ceria nanoparticles suspensions on fresh water micro-algae

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When assessing the hazards properties of nanomaterials in the environment, the main research challenges are numerous. Firstly, determining if nanomaterials are more or less toxic than the bulk forms of the same materials and the extent to which toxicity is governed or influenced by the physico-chemicals properties of the nanoparticles. Secondly, it appears necessary to study the effect of nanomaterials and nanoparticles throughout their life cycle including both initial forms and physico-chemically modified form (i.e. aggregated or agglomerated forms) resulting from an ageing process.

Our work focused on the effect of commercial ceria nanoparticle (nCeO2) suspensions, towards freshwater micro-algae assessing the effect nCeO2 suspensions with different agglomeration/aggregation state obtained by using an artificial ageing process. Both ageing and non-ageing nCeO2 suspensions were fully characterized using dynamic light scattering (ZetaSizer, Malvern Instruments) or laser diffraction (MasterSizer, Malvern Instruments) and transmission electron microscopy (TEM). In addition, the interaction between NPs and algae were investigated using flow-cytometry and environmental scanning electron microscope technique (E-SEM).

The results obtained showed that the algae growth inhibition was similar after exposure to non-ageing or ageing nCeO2 suspensions. The results obtained from flow-cytometry and E-SEM proved that the ceria NPs are able to tightly entrap the algae cells, which could in part contribute to the effect recorded. Those results also support the fact that aggregation or agglomeration has a few influences when focusing on the standardized algae ecotoxicity test. Moreover by comparison to our previous studies performed with other ceria suspensions, it was shown that the primary particle size and consequently the particle surface area is a relevant parameter in assessing the ecotoxicity of nanoparticles.

EP03C-1

Bioavailability of carbon nanotubes to aquatic organisms of different trophic levels and the consequences of CNT-cell interactions to vital functions

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Multi-walled carbon nanotubes (CNT) were synthesised using 14C-benzene in order to quantify bioaccumulation of this nanomaterial in different aquatic organisms. Next to uptake and elimination, transfer of CNT along the food chain, the influence of the presence of sediment or dissolved organic carbon (DOC) on its bioavailability, and distribution of incorporated CNT to different fish tissues were investigated. Radioactivity was detected in algae, daphnids, blackworms, and zebrafish, which had been exposed to dispersed 14C-CNT material via the water (1 mg/L). Furthermore, single tubes and small agglomerates were visualised by means of transmission electron microscopy in algal cells, branchial erythrocytes of fish, and gut epithelial cells of daphnids, worms, and fish. Transfer of CNT from the fish gills to the blood current was confirmed by the presence of radioactivity in blood samples and gonads. However, CNT were not detected in the brain of zebrafish, which indicates that they do not pass the blood-brain barrier. CNT were not bioavailable for blackworms when spiked to the sediment. Similarly, ingestion of CNT-containing prey by daphnids (CNT-spiked algae) or by zebrafish (CNT-spiked worms) resulted in lower accumulation in the predator compared to after uptake of equal amounts via the water phase. Although DOC was shown to keep prepared dispersions more stable over time, its presence had no influence on CNT bioavailability. The consequences of the observed interactions of CNT with cells, following water exposure of different organisms, regarding possible damage to vital functions are investigated at the moment. By now, CNT-associated alterations in the cell

biochemistry of exposed algae were observed by attenuated total reflection Fourier-transform infrared spectroscopy. Furthermore, the condition factor of fish that had taken up CNT during four days, and were daily fed for six days afterwards, was significantly lower compared to the one of control organisms. This might indicate that digestion of food is hindered by the observed presence of CNT material in gut epithelial cells. Longer tests with repeated exposure periods are currently performed to verify this finding. The combined results of the present fate and effect studies deliver conclusive insights that can be used to assess the possible risks of CNT release to the aquatic environment.

EP03C-2

Short-term toxicity of silver nanoparticles on litter-associated fungi and bacteria from streams

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Current knowledge of AgNP toxicity is mainly based on laboratory studies with single species, which might not reflect their effects in natural environments. Therefore, it is essential for realistically assessing AgNP effects to apply approaches that take the ecological complexity and variability of natural communities and ecosystems into account. Litter-associated fungi and bacteria provide suitable model systems for community ecotoxicology because they play a fundamental role in the functioning of various ecosystems. They effectively degrade plant litter and produce substantial amounts of biomass that is subsequently channeled to higher trophic levels. This study assessed the short-term toxicity of AgNP on litter-associated stream fungi and bacteria in comparison to that of AgNO3 (Ag+). Prior to use, the nanoparticles were characterized in terms of size, surface charge and aggregation behaviour. Toxicity of AgNP in comparison to Ag+ was determined in inhibition tests based on a broad set of functional parameters. The specific sensitivity of fungal communities was assessed as sporulation rate and 14C-acetate incorporation into ergosterol. The specific sensitivity of bacterial communities was assessed by determining rates of 14C-leucine incorporation into protein. Moreover, potential extracellular enzyme activities (phosphatase, -glucosidase and leucine-aminopeptidase), and respiration were measured to evaluate effects on overall microbial activity. Toxic effects of AgNP were variable, depending on the functional parameter used, and distinct from Ag+ toxicity. No inhibitory effect of AgNP on microbial respiration was observed, whereas Ag+ caused an inhibition. Both AgNP and Ag+ exposure stimulated potential phosphatase activity and reduced potential leucine-aminopeptidase activity. -Glucosidase activity was stimulated by AgNP but reduced by Ag+. Finally, bacterial and fungal growth and sporulation were more strongly inhibited by AgNP than by Ag+. These findings suggest that dissolved ionic Ag (i.e. AgNO3) does not fully explain the observed toxicity of AgNP on litter-associated fungi and bacteria in streams. Overall, our study confirms the need to consider functional diversity using complementary indicators for ecotoxicological investigations on short-term AgNP toxicity.

EP03C-3

The effect of ageing on the bioavailability and ecotoxicity of ZnO-NP, bulk ZnO and ZnCl2 in natural soil

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Releases of metal nanoparticles into soils are expected when nanoparticles pass the sewage treatment plant or end up in sewage sludge. Concern has risen about ecotoxicological effects of nanoparticles. Short-term toxicity tests and bioaccumulation studies on zinc oxide nanoparticles (ZnO-NP) have already been performed with isopods, earthworms and springtails. Little information is available on the long-term fate and effects of ZnO-NP in soils.
Here the behaviour of ZnO-NP, bulk ZnO and ZnCl2 was studied in natural soil to evaluate the influence of aging time on chemical speciation and stability of the nanoparticles. Dissolution and ecotoxicity to the springtail *Folsomia candida* was determined for the three Zn forms after three, six and twelve months.
Zinc concentrations in the soil pore water ranged from 1.85 to 12.6 mg Zn/l in freshly spiked soil with ZnO-NP and this increase was found to be linear. Porewater concentrations after three, six and twelve months increased in a non-linear manner with increasing soil concentrations for ZnO-NP and bulk ZnO. Zn concentrations in the pore water increased with time, but peaked at intermediate concentrations. The highest Zn concentrations measured after one year ageing were 67.1 and 66.5 mg Zn/l for ZnO-NP and bulk ZnO, respectively. Zn concentrations in pore water, collected from aged soils with $ZnCl_2$, increased with exposure concentration, but also with time. ZnO-NP and bulk ZnO were toxic in freshly spiked soil with no significant difference between the two ZnO powders. No effect on Collembolan survival or reproduction was found in three, six or twelve months aged soil with ZnO-NP and bulk ZnO. The toxicity of $ZnCl_2$ decreased with time as shown by the three-fold increase in EC50 value after three months ageing.
The release of Zn ions from ZnO-NP was reduced at high spiking concentrations. Sorption and agglomeration may inhibit dissolution and increased sorption may take longer due to the physical and chemical properties of nanoparticles. An increasing solubility throughout one year was observed, although at very low rate when based on total Zn concentrations.
Several studies show that toxicity to soil organisms is related to the free ions released from metal nanoparticles. Our toxicity tests show that an ageing period of three months or longer is able to reduce toxic effects on survival or reproduction to *F. candida*.

EP03C-4

Effects of C60 nanoparticles on Lumbricus rubellus earthworms: from gene expression to population dynamics

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In the present study the effects of C_{60} exposure on *Lumbricus rubellus* earthworms was assessed at different levels of biological integration. Two in vivo experiments were carried out, with the same exposure levels of C_{60} (0, 15 and 154 mg C60/kg soil). In the first experiment adult earthworms were exposed for four weeks and in the second experiment the offspring was exposed lifelong. Effects on the individual level were assessed. C_{60} exposure caused a decrease of reproductive success in the four weeks exposed earthworms and offspring exposed to C_{60} demonstrated lower juvenile survival and growth rate. These effect markers were used to model consequences at the population level. The population model showed that C_{60} exposure could affect population growth rate and life stage distribution. In order to relate these observations to potential modes of action, sub-lethal responses were also studied for these earthworms. At the tissue level, histological examinations demonstrated concentration-dependent effects in both experiments. Damage was noted at the cuticle (accompanied with injuries to underlying tissues) as well as at the gut epithelium. At the molecular level, gene expression analysis was performed for several selected genes. The general stress marker heat shock protein 70 (HSP70) and the cytokine-like coelomic cytolytic factor 1 (CCF-1) were both affected by C_{60} exposure. As an effect on immune response (CCF-1) was noted, in vitro experiments with immune cells were performed. Coelomocytes, extracted from adult earthworms and exposed in vitro, showed decreasing survival and phagocytic activity with increasing C_{60} concentrations. Furthermore, C_{60} will be characterized in the exposure media, to assess actual exposure. In conclusion, this study demonstrates the impact of C_{60} exposure at the molecular, cellular, tissue, individual and population level. Further research is needed to identify the precise mode of action of C_{60} exposure to earthworms.

EP03C-5

Effect of Cu-NPs versus Cu-salt in Enchytraeus albidus - survival, reproduction, avoidance, oxidative stress biomarkers, energy allocation and gene expression - a systems biology approach

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Environmental effects of Cu-nanoparticles (Cu-NP) are little studied in terrestrial ecosystems. In the present study, the toxicity of Cu-NPs on the enchytraeid Enchytraeus albidus is compared to the toxicity of a copper-salt (CuCl2). Traditionally, effect assessment is made at the population level [1]: survival and reproduction. Recently, a cDNA microarray has been developed, which allows toxicogenomic studies. The effect was studied including various endpoints towards a systems biology approach: survival, reproductive output, avoidance behaviour, oxidative stress biomarkers, energy budget (lipids, proteins, carbohydrates) and gene expression. The results from survival, reproduction and avoidance showed that Cu-NPs were more toxic to E. albidus than the same concentrations of the CuCl2-salt. The physic-chemical analysis of the particles indicated that only a small fraction was released as ions. Hence, the results indicated a nanoparticle-specific effect - lower reproductive output and higher avoidance. This was observed as 2- 8 fold (significant) lower ECx values for Cu-NP exposed organisms compared to CuCl2 exposed organisms. These results indicated a nanoparticle-specific effect. Oxidative stress biomarkers showed that both salt- and nano-copper caused oxidative stress and damage to E. albidus, as confirmed by LPO levels, and effects could be discriminated between the copper forms. Nevertheless and despite the visible discrimination between nano and the salt form (time and exposure dependent), there was no single or a set of biomarkers that provided the best discrimination. The energy allocation levels showed clear differences between worms exposed to control soils and those exposed to Cu for 3 weeks, but no difference after 6 weeks exposure. There was no apparent difference between the impacts of the two Cu exposure forms. The number of differently expressed genes (DEG) decreased with increasing concentration for CuCl2 exposure, whereas for Cu-NP, the number did not change. The number of common DEG decreased with increasing concentration. Differences were mainly related to transcripts involved in energy metabolism (e.g. monosaccharide transporting ATPase, NADH dehydrogenase subunit 1, cytochrome c). Overall, our results indicated that Cu-salt and Cu-NP exposure induced different gene responses. All results pointed to a

nanoparticle-specific effect, and not due to Cu-ions released from the particles, as also indicated by the ISE measurements.

EP03C-6

Long-term effects of sewage sludge spiked with Ag-NP on soil microorganisms

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Due to their antibacterial and antimicrobial properties silver nanoparticles (Ag-NP) are widely used, e.g. in textiles, medical applications or cleaning products. This means that Ag-NP will inevitably enter wastewater treatment plants (WWTP). In the literature, research is reported on the fate and behavior of Ag-NP in WWTPs. Burkhardt et al. (2010) determined that nearly 95% of silver is bound to the sewage sludge whereas around 5% leaves the WWTPs with the effluent in ecologically negligible concentrations. In the sewage sludge silver ions are transformed to silver sulphide which precipitates. In Germany and other countries sewage sludge is used as a fertilizer in agriculture. Therefore, the goal of the present project was to determine long-term effects of sewage sludge spiked with soluble Ag (silver nitrate) and Ag-NP on soil microorganisms over a period of 180 days. We used sludge from a local WWTP and spiked it with silver nanoparticles - NM-300K from the OECD Sponsorship Programme - and silver nitrate. The test soil was the medium acidic and slightly humic loamy sand "Refesol 01A". Silver concentrations of 1.6 and 3.4 mg/kg d.m. soil for NM-300K and 2.1 and 4.0 mg/kg d.m. soil for silver nitrate were obtained. To investigate potential effects on soil microorganisms standardized test systems were used. Ammonium oxidation (ISO 15685) and carbon transformation tests (CTT; OECD 217) were performed analyzing samples at day 32 /60 / 100 and 180. Two replicate batches were used for each concentration. A control without sludge and one with non-spiked sludge served as reference. Effects on ammonium oxidation or respiration (CTT) of the microorganisms based on Ag-NP or silver nitrate were not observed after 32 days. Referring to the control with sludge we obtained an inhibition of the microbial respiration activity for the highest concentration of silver nitrate after 60 days, whereas ammonium oxidation showed no effect. After 100 days both concentrations of NM-300K and silver nitrate resulted in a comparable respiration inhibition of around 30%. The ammonium oxidation test showed a statistically significant dose-dependent inhibition for NM-300K and silver nitrate. Results for day 180 are not yet available. Our results indicate that Ag-NP and silver nitrate can become bioavailable after the degradation of sewage sludge and can cause an inhibition of the soil microorganisms.

EP03D-2

An approach to determine appropriate dose metrics for nanomaterials

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Exposure limits for chemicals are traditionally derived from studies in which increasing doses of the substance are administered. For soluble substances, a unique measure of the dose is the total mass of the substance administered or, equivalently, the total number of molecules administered. Consequently, exposure limits are generally based on mass concentrations, such as X mg of chemical substance Y per kg soil.
With nanotechnology a new array of often insoluble chemical entities has made its appearance. The different characteristics of nanomaterials (e.g. size, shape, polymorph form of the crystall structure) all may determine their toxic potential. For example, daily intake of X mg of nanomaterial (NM) with particle size d1 may be more toxic than X mg of the same nanomaterial with particle size d2. In other words, for NMs information on the administered mass of the chemical substance alone may not be a sufficient description of the dose that determines a particular response. As a result, the question arises of what dose description to use when setting exposure limits for NMs. It has been speculated that exposure limits based on particle numbers, such as used for particulate matter, may be more appropriate, while others advocate surface area.
An adequate dose metric for NMs should describe all relevant characteristics that are necessary to explain differences between responses. A minimal criterion for an adequate dose metric is that the dose metric should be able to discriminate doses with different responsesw. In its most complete form, the dose of a NM can be described by a (distribution) function that specifies the number of particles in the ensemble with specific characteristics. For example $PN(d,\zeta,\alpha)$ may give the number of particles N with a diameter d, surface potential ζ and crystal structure α.
Ideally, a dose metric should be as concise as possible, with as few dimensions as possible. A reduced dose metric (for example requiring only information on administered total number of particles, or total surface area) would be pragmatic for risk assessment purposes, since only one exposure limit would have to be derived for various NMs of the same substance. However, a priori there is no reason why such a reduced dose metric should exist. In this contribution, we present a method to determine whether a reduced dose metric for (a class of) NMs exists. As an illustration, the method is applied to results from experiments with various NM published recently.

EP03D-3

Assessment of environmental risks of nanomaterials throughout the product life cycle

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Estimation of exposure due to release into the environment represents a key challenge in determining nanomaterials safety. It is therefore important to understand to what extent existing risk assessment approaches and tools can be applied or will require modification to take account of the particular properties of nanomaterials. The present work aims to identify methodologies and tools to assess and manage the environmental risks due to manufactured nanomaterials, identify appropriate risk assessment methods, and develop improved methods for risk assessment. Some currently available nano-specific support tools are evaluated, in order to identify gaps where either risk assessment methods and/or input data are insufficient.
A significant problem is that there still remain many outstanding questions regarding the behaviour of nanomaterials in the environment, which could differ from that of materials of similar composition in bulk form. Reducing the level of these uncertainties is essential to proper risk assessment. There is thus an urgent need for reliable data on the physico-chemical properties, toxicokinetics and degradability of nanomaterials to understand their transport, persistence and fate, and exposure potential in the environment. However, there is still a lack of basic information on the possible release routes for nanomaterials during production, use and final disposal or recycling. For this reason, it is necessary to improve our present knowledge on the release of nanomaterials from products during all phases of their life cycle.
The NanoSustain project is developing innovative solutions for the sustainable use, recycling and final treatment of nanotechnology-based products. Experimental work is carried out to investigate the potential for release of nanomaterials from products during industrial operations, such as sanding and grinding, and at the end of life during disposal by incineration and recycling by melting. Data obtained from such experiments should help reduce the level of uncertainly in the risk assessment

EP03D-4

A weight of evidence approach for ranking and prioritization of occupational exposure scenarios for engineered nanomaterials

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It has been recognized that substantial limitations and uncertainties make the conventional risk assessment of chemicals infeasible to apply to engineered nanomaterials (ENMs) today, which raises health and safety concerns. The present deficit of quantitative exposure measurements and nano-specific exposure models will lead in the near term to uncertain and largely qualitative exposure estimations, which may fail to support proper risk assessment and management actions. In this context it is necessary to develop well-validated, easy-to-use approaches for early occupational and consumer safety evaluation [1]. A variety of such tools already exist: e.g. the Swiss Precautionary Matrix for Synthetic Nanomaterials, the Dutch Stoffenmanager Nano, the Danish NANOSAFER, the French Anses system. This is currently a dynamic area of research and several tools are still under development. However, with few exceptions, they all produce qualitative results.
In this context we propose a quantitative model for relative ranking and prioritization of occupational exposure scenarios (ES), specifically tailored for ENMs. The tool is based on a scoring weight-of-evidence method and it uses expert judgement to estimate exposure potential of nanomaterials in the workplace. The conceptual structure of the approach has been defined on the basis of state-of-the-art exposure models for conventional chemicals like the Advanced REACH Tool (ART) and for nanomaterials, such as the Stoffenmanger Nano. In order to apply the tool we used data from of the EU FP7-funded NANEX project. Using the model we obtained rankings for 3 groups of inhalation occupational ES and, in order to validate our results, we compared them with parallel rankings of the measured exposure concentrations of the materials, reported for the same scenarios. The results show that the WoE-based model slightly overestimates the exposure in most cases, which proves that it is a conservative approach. In order to test the stability of the model, probabilistic Monte Carlo sensitivity analysis has been applied and it shows that the tool always performs in a stable manner in regard to variations in the input data.
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EP03D-5

Comprehensive environmental assessment of nano-based self decontaminating surfaces

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Engineered nanoparticles are being exploited for a wide variety of military applications. Material science research into the development of new engineered nanoparticles

is far outpacing environmental and human health and safety research, yet the health and safety data are critical for acquisition decisions, regulatory decisions, worker safety, product use and disposal, and public acceptance of nanoparticle-containing products. Traditional life cycle analyses address key steps in nanoparticle synthesis, use, and disposal, but lacks specific information regarding fate and effects in the environment. Conversely, traditional environmental risk assessments address fate and effects of chemical stressors in the environment, but only consider chemicals at specific environmental sites and not throughout the chemical's life cycle. We are developing and applying the comprehensive environmental assessment (CEA) approach, detailed by Davis (2007), which combines life cycle analysis parameters (e.g., manufacture, storage, use, disposal) with traditional risk assessment parameters (e.g., characterization, exposure, effects, assessment) to understand of nanoparticle exposure and effects in different environmental settings. The application of this approach is demonstrated through a case study examining novel nano-based reactive surfaces; a UV-light activated technology. Specifically, we have examined the residue of aged coatings following UV exposure; the most likely release of nanoparticles from this specific nanotechnology. Results of this study represent one of the first documented releases of a nano-sized particulate from a nano-enabled technology. The use for CEA for engineered nanoparticles will improve acquisition, risk, and regulatory decision making and management prior to any unforeseen adverse environment, health, and safety (EHS) events that could dramatically impact the use of these revolutionary new materials.

EP03D-6

Multimedia environmental fate models for engineered nanoparticles - a case study of nano-TiO2 in the Rhine River

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With the increased production and use of engineered nanoparticles (ENPs) their release to the environment becomes inevitable. Multimedia fate models are valuable tools to assess exposure levels of pollutants in different environmental compartments, both at a regional as well as at a global scale and have the potential to be used in a pro-active risk assessment of ENPs. However, whereas multimedia environmental fate models are well established for organic pollutants, the field of environmental fate modelling is still in its infancy for ENPs. Due to the fundamentally different properties of ENPs compared to normal (i.e. low-molecular weight) organic pollutants, it is necessary to completely revise the process descriptions used in the models and to adjust them to account for ENP-specific properties. Here we present a new framework of multimedia fate models to describe the fate and behaviour of ENPs in aqueous environments and predict exposure levels of ENPs.

As a first case study and illustration of our new multimedia fate modelling concept for ENPs we present a study on TiO2 nanoparticles (NPs) in the Rhine River. The core of this model is the parameterization of ENP-specific processes. A key process governing the environmental fate of ENPs in aqueous environments is the heteroaggregation of ENPs with naturally occurring suspended particulate matter (SPM) in the nano- and micrometre size range. Deposition of free ENPs by gravitational settling and of ENPs attached to SPM determine the distribution of ENPs between water and sediment compartments.

Our model enables the prediction of steady-state concentrations of TiO2 NPs along the course of the Rhine River. Varying the parameters affecting the heteroaggregation process allows us to compare their relative importance on the overall fate and transport of TiO2 NPs in the model system. Overall, the sediment compartment always represents the main reservoir of TiO2 NPs, but the concentration profile and transport potential of TiO2 NPs in the water compartment strongly depends on the heteroaggregation attachment efficiency, $C_{het\text{-}agg}$, and the characteristics (size, density and concentration) of the SPM.

EP04 - Greener nanotechnology, an integrative approach to an emerging technology

EP04-1

Green nanotechnology challenges and opportunities

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Nanotechnology is an emerging field, for which there is an unusual opportunity to use science, engineering and policy knowledge to design novel products that are benign as possible to human and environment health. Recognition of this opportunity has led to the development of the "green nanoscience"concept.

Developing an Action Agenda

Green nanotechnology has been making great forward progress, but the challenges it has encountered point to an agenda of actions where involvement by the scientific research community, industry and government is crucial to supporting a more rapid and effective commercialization of green nanotechnology.

The agenda is based on input from experts in green who participated in the GN10 and GN11 Conferences in 2010 and 2011. The first, and most pressing need is for more and better analysis and characterization tools, which are required to support the rest of the agenda. The second item of the agenda, improved mechanistic understanding, is a key part of the foundation for developing green nano design guidelines. Finally, new regulations, as well as outreach to regulators must be based on the analysis, understanding, and design concepts that are the result of the first three items.

The Agenda

1. Discover, uncover and provide key analysis and characterization tools,
2. Develop, characterize and test precision-engineered nanoparticles for biological and toxicological studies needed to guide greener design,
3. Investigate and understand reaction mechanisms to support more efficient and precise synthesis and production techniques,
4. Develop design guidelines for green nanomaterials,
5. Definition of green criteria for new nanomaterials for fast-track approval by the US EPA, and
6. Education and outreach to regulators to ensure regulatory structures for green nanotechnology reflect accurate knowledge of their intended uses and potential impacts.

Nanotechnology does not have to follow the typical path of many past innovations in the chemical industry that, despite providing significant benefits, also turned out to have unanticipated costs to human and environment health. The development and commercialization of viable green nanotechnologies is difficult, and it will require effort from the scientific, research and government communities. But there is a pathway forward, and concrete actions that could construct a solid foundation for a profitable and environmentally sustainable future for nanotechnology.

EP04-2

Rapid in vivo assessment of the nano/bio interface to guide safer nanomaterial design

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The rapid rate of discovery and development in the nanotechnology field will undoubtedly increase both human and environmental exposures to engineered nanomaterials. Whether these exposures pose a significant risk remains uncertain. Despite recent collective progress there remain gaps in our understanding of the nanomaterials physiochemical properties that drive or dictate biological responses. The development and implementation of rapid relevant and efficient testing strategies to assess these emerging materials prior to large-scale exposures could help advance this exciting field. I will present a powerful approach that utilizes a dynamic in vivo zebrafish embryonic assay to rapidly define the biological responses to nanomaterial exposures. Early developmental life stages are often uniquely sensitive to environmental insults, due in part to the enormous changes in cellular differentiation, proliferation and migration required to form the required cell types, tissues and organs. Molecular signaling underlies all of these processes. Most toxic responses result from disruption of proper molecular signaling, thus, early developmental life stages are perhaps the ideal life stage to determine if nanomaterials perturb normal biological pathways. Through automation and rapid throughput approaches, a systematic and iterative strategy has been deployed to help elucidate the nanomaterials properties that drive biological responses.

EP04-3

Ecotoxicology in nanoremediation: n-TiO2 nanoparticles increase Cd bioaccumulation and toxicity in Mediterranean mussel Mytilus galloprovincialis

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The present study investigates the interaction of TiO2 NP with Cd and how the presence of n-TiO2 might affect Cd bioavailability and toxicity in a model marine species, the Mediterranean mussel Mytilus galloprovincialis. n-TiO2 is an efficent photocatalyst largely used in several technological applications including water and aqueous wastes remediation. Cd is one of the most toxic metals of interest for remediation. Both compounds have been quite extensively studied in terms of toxicity in the Mediterranean mussel showing distint pathways of cellular interaction and biological targets. Mussels have been in vivo exposed for 24h and 7 days to n-TiO2 (1mg/L) and Cd (0,1mg/L) in single and co-incubation. The documented adsorption of Cd onto n-TiO2 has been confirmed also in our study; the presence of n-TiO2 enhanced Cd bioaccumulation in mussel's whole soft tissue and increase Cd citotoxicity (reduction of Neutral red retention time in hemocytes). A significant disruption/reversion of Cd capacity to increase the efflux functionality of ABC tranport proteins (most probably involved in Cd-resistance) has been also observed in presence of n-TiO2. Our results indicate that specific NP applied for remediation purposes might exert indirect toxic effects to the biota by affecting both bioavailability and toxicity of the removed toxic contaminant. The present study will promote nano-ecotoxicology as a new strategy in order to develop eco-friendly nanomaterials for specific technological application as nanoremediation.

EP04-4

Nanoparticle properties affecting embryotoxicity: toward a design of safer nano-Zinc oxide

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The impact of nano metal-oxides on human and environmental health is predicted to be increasing. Among metal oxides, nano ZnO (nZnO) is retained one of the most dangerous. Recently nZnO has invaded the market for its UV protective and antibacterial properties, that make it suitable for a wide range of application for functional coating formulations to protect wood, plastics, textiles from UV and microbial degradation. Previous data already showed that nZnO has a powerful embryotoxic potential on X. laevis and that it was able to mainly affect gut development. It was clearly demonstrated that nZnO produced severe lesions at the intestinal mucosa and potentially cross the gut barrier reaching the underlying tissues. In this work we used Xenopus laevis embryos to characterize the embryotoxic and teratogenic potential of nZnO according to the modulation of NP size and surface charge, as well as to the irradiation conditions. To optimize the stability of the NP suspensions and to achieve useful NP-surface functionalization, we worked in strict connection with a private nanotech company with both R&D and commercial activities. The purpose was to provide mechanistic data on nZnO ecotoxicology and to suggest criteria to design safer Zinc oxide NPs.
We demonstrated that nZnO-induced embryotoxicity was mediated by NPs' own reactivity rather than ion dissolution and that it is strongly associated with the modality of the biological interactions at the nano-level, which at last depend upon the physical and chemical NP surface properties. These properties are also at the base of the induced oxidative potential by nZNO, which is also very efficiently modulated by light irradiation. Finally NP dimension, and especially surface charge, played a crucial role in determining the embryotoxic potential and the intestinal translocation and lesions of nZnO.
The present results showed how a comprehensive knowledge of the nZnO physical and chemical properties, affecting the interactions at the bio-interface, may contribute to make nanotoxicology a predictive science and may help chemists and material scientists in the design of safer NPs.

EP04-5
Greener nanomanufacturing: toward low-waste and high-yield synthesis of monodispersed metal oxide nanoparticles
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Metal oxide nanoparticles have attracted much attention due to their wide variety of applications, such as catalysts, energy storage materials, solar cells, light emitting diodes, sensors, transparent electrodes. For practical applications, syntheses should be greener, with low waste and high yields, in order to avoid future environmental health and safety risks and to prevent material resource depletion. If industry plans to use a broad array of metal oxide nanoparticles and employ hundreds of different chemicals to synthesize, we may have to develop a large number of new syntheses and attempt more than hundreds of different toxicological assessments because the specific policies for each environmental impact will be considered including combination of chemicals. To avoid such complicated risks, we require a few generalized synthesis methods of nanoparticles having both technical and environmental performance in order to simplify production and toxicological assessments.
We propose a synthesis route through an pseudo-first-order catalytic esterification using only oleyl alcohol and oleic acid, derived from non-toxic natural oils, as solvents, reagents, and surfactants during a one-step synthesis of metal oxide nanoparticles. This greener approach simplifies the assessment of polution and toxicity.
We have synthesized metal oxide nanoparticles at 230 °C or less by using an pseudo-first-order catalytic esterification. This approach offers much lower temperature and shorter reaction time than the other methods to produce monodispersed metal oxide nanoparticles with high yield. The produced byproducts are water and oleyl oleate. The oleyl oleate could be recycled to oleyl alcohol and oleic acid again by using hydrolysis.
All of metal oxide nanoparticles are monodispersed and show no aggregation. The average diameters of monodispersed In_2O_3 and ITO nanoparticles are 7.2 nm and 6.3 nm in diameter (with 89 % and 87 % dispersities within ± 1 nm differences from the average diameter), respectively. The yield of nanoparticles at 230 °C is greater than 90 %.
The pseudo-first-order esterification offers a number of advantages for metal oxide nanoparticle synthesis because it is rapid, produces high yields, permits precise doping, and minimizes waste. We will demonstrate syntheses of several different metal oxide nanoparticles.

EP04-6
Cadmium-free quantum dot nanocrystals for lighting and displays
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Quantum dot (QD) semiconductor nanocrystals have been considered in a broad range of applications, from biological tagging to LEDs, lasers, displays, lighting and solar cells. In their photoluminescent mode of operation, QDs are currently in lighting products, and have the promise to be in liquid crystal display products in the near future. In electroluminescent mode, quantum dot light emitting devices (QLEDs) are an emerging class of thin-film hybrid organic-inorganic structures that can potentially achieve best-in-class performance amongst large-area emissive light sources [1]. Market research projects world-wide QD production to increase from ~1kg today to ~1000 kg by 2015. Should solar cell applications of QDs come to fruition, QD production could further increase to several hundred metric tons.
Historically, QDs are made out a semiconductor core comprising Cadmium Selenide (CdSe). This is due to 1) the early discovery in the 90's of facile synthetic methods for the lab scale production of QDs of this material, 2) the visible wavelength compatibility of this quantum confined system, 3) the general ease of II-VI semiconductor synthesis and 4) the lack of an equally facile and visible-emitting alternative semiconductor. The CdSe system has been ideal for scientific study, and has even led to early product launches most notably in lighting where the environmental net benefit is easily demonstrated. However, due to the combination of known Cadmium toxicity, world-wide regulations, and consumer sentiment, it is desirable to make available an alternative for Cadmium containing QD materials which are Cadmium-free. This green-chemistry guided research and development effort is an ideal example of how nanomaterials afford the opportunity for materials design not just for performance, but simultaneously to minimize the potential for environmental impact. However, when assessing environmental impact, it is crucial to utilize a cradle-to-cradle view of the material's lifecycle.

EP05 - Non-target analysis and identification of toxicologically significant emerging pollutants

EP05-1
Prioritising non-target identification in wastewater effluent: from picking peaks to programs!
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Non-target analysis of wastewater effluent poses many challenges, including a multitude of peaks and potential interferences of matrix constituents with non-target analysis. However, these samples also offer much valuable information about emerging contaminants in the environment, especially those resulting from urban sources. Here, we detail the results of non-target analysis and prioritisation in several wastewater treatment plant effluents from around Switzerland. The program enviMass was used to perform both target and non-target identification of all samples. The list of non-target masses from all samples were then compared and prioritised according to intensity (and thus, approximately concentration in the environment). This way, thousands of non-target peaks could be reduced to a much smaller list of interesting masses for data-dependant MS/MS analysis to provide fragmentation information for identification of the non-targets. Then, using "the more information the merrier"approach, we gathered as much information as possible from the analysis to hone in on the most likely molecular formulas and thus corresponding candidates. Programs used here included MOLGEN-MSMS (formula calculation), MetFrag and MetFusion (database query and fragmentation prediction) and Mass Frontier (rule-based fragmentation prediction), as well as several different calculations for partitioning and retention properties of compounds. Here, we show how the enviMass workflow allowed a quick and effective selection of peaks of interest and comparison of samples, while the combination of features from many programs improved the chances of non-target identification. While the confirmation of tentatively-identified non-targets is very dependent on the analytical information, the number of possible candidates and the availability, we show how the combination of a few key programs can maximise the use of analytical information and streamline these efforts greatly.

EP05-2
Screening and toxicological evaluation of organic micro-pollutants in the Rhine and Meuse river basins
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The rivers Rhine and Meuse serve as drinking water source for millions of people in Europe. In these waters, rapid improvements in chemical and bioanalytical techniques have led to the discovery of all kinds of emerging contaminants at very low concentrations. Some studies also reported the presence of traces of emerging contaminants in drinking water samples. Dutch drinking water companies therefore intensively investigate their water sources for the presence of emerging contaminants and their fate during treatment processes.
Because thousands of compounds are present in the aquatic environment, it is impossible to detect their presence via target analysis in standard monitoring programs. Non-

target screening is therefore used as an important additional tool to obtain information on the types of organic contaminants that are present in the aquatic compartment. It functions not only as an intake monitoring tool, but also as a safety net for new or unknown compounds which are not included in the target analyses.
In this study, sensitive GC-MS non-target screening methods were developed (up to LOD 1 ng/L) and applied to different locations in the Dutch part of the Rhine and Meuse river basins in 2010 and 2011.
A first goal of the study was to examine which known and unknown compounds are found in the Rhine and Meuse. By mapping the organic pollution in the river basins, and following the trends of pollution over the year, an integrated picture on the presence of organic pollutants in place and time was obtained. Up to 400 different compounds were found in the water samples. Screening results for the Rhine and Meuse indicated that although there are differences in the occurrence of compounds between the sources, some compounds appear to be widespread (e.g. Surfynol 104 and 5-methyl-1H-benzotriazole). Industrial compounds and flame retardants comprise the largest groups of compounds.
A second goal of the study was to investigate whether there are compounds present that are potentially of concern for the drinking water production. To this aim an integrative ranking system was developed in which the identified compounds were sorted according to aspects as their human toxicological risk, frequency of detection, persistence in drinking water treatment and associated public concern. A top ten of contaminating compounds that are most relevant for drinking water production from the rivers Rhine and Meuse was compiled. This list will be nominated for political action.

EP05-3
Integrated characterization of a mutagenic waste water treatment plant effluent combining advanced screening techniques and biological assessment
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Increasing numbers of emerging contaminants have been detected in surface water over the last decade. Waste water treatment plant (WWTP) discharges were identified as main sources of these chemicals and related effects in the environment [1, 2]. For this reason, effluents of WWTPs were assessed to gain knowledge about discharged chemicals, their concentrations and their effects on the environment. Linking the discharged chemicals to effects or identifying the substances revealing measured effects is the main challenge for the assessment of WWTP effluents.
To meet this challenge in the present study an integrated biological and chemical approach is applied to identify genotoxic and mutagenic compounds in an effluent of a WWTP treating industrial and municipal wastewater as well as contaminated groundwater. The approach combines extensive target and suspect screening with biological effect assessment in vitro by Ames fluctuation assay on the basis of a series of grab samples taken over several weeks and in vivo by fish caging.
For chemical characterization of WWTP effluent, a target screening on 300 environmental relevant chemicals and a suspect screening on 1800 chemicals applied or produced at the industrial site was performed. Six site specific suspects were identified. Concentration trends of identified targets and suspects have been recorded, focusing particularly on indicator compounds for industrial and domestic waste water, as well as for groundwater co-treated in this WWTP. In the biological assessment, nine of eleven methanol extracts and all BR extracts exhibited mutagenicity in the Ames fluctutation assay. Based on this data, relationships between target and suspect substances and mutagenicity of samples will be presented.
[1] Fent K, Weston AA, Caminada D. 2006. Ecotoxicology of human pharmaceuticals. Aquat Toxicol 76:122-159.
[2] Claxton LD, Houk VS, Hughes TJ. 1998. Genotoxicity of industrial wastes and effluents. Mutat Res 410:237-243.

EP05-4
Identification of biotransformation products (BTPs) formed in freshwater crustaceans
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Freshwater crustaceans, *Daphnia magna* and *Gammarus pulex* are widely distributed in watersheds which are contaminated by anthropogenic compounds. After uptake of organic contaminants the organisms possibly form biotransformation products (BTPs) by means of enzymes involved in detoxification. Little is known on BTPs in crustaceans and their relevance to explain chemical fate and toxicity. In the present study, *D. magna* and *G. pulex* were exposed to selected organic contaminants and then their BTPs were identified through the suspected/non target screening methodology by using high resolution LC-tandem mass spectrometry. The structure elucidation of BTP was performed through MS/MS spectra interpretation with a fragment prediction tool. In addition, the BTP prediction tools currently available were evaluated in terms of the feasibility of application to crustaceans.As the results, various reaction mechanisms such as N-dealkylation, O-dealkylation, N-oxidation, hydroxylation, epoxidation and glycine conjugation lead to the BTPs for irgarol, terbutryn, tramadol, or venlafaxine. Irgarol and terbutryn which both have triazine moiety showed similar biotransformation pathways. No BTPs were identified for valsartan and clarithromycin so far. This may be caused in general by the lower bioaccumulation of the relatively polar and ionized compounds. More BTPs were identified for *G.pulex* compared to *D.magna*. A number of dealkylation and oxidation products were successfully predicted by the prediction tools. However, the manual prediction based on biochemical knowledge was most successful.

EP05-5
Input and fate of contaminants in surface waters observed by suspect and nontarget screening with LC-Q-TOF-MS
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The increasing availability and sensitivity of LC-MS systems has widely opened the analytical window for polar analytes, the so-called new emerging contaminants. These are increasing numbers of compounds from pharmaceuticals and personal care products, herbicides, fungicides and industrial chemicals. LC with high resolution mass spectrometry enables to perform screening approaches to detect a considerable number of known and unknown analytes in complex samples.
In this work we will show the application of target and nontarget screening with LC-electrospray ionization-quadrupole-time-of-flight-mass spectrometry (LC-ESI-Q-TOF-MS) combined with computerbased data evaluation based on statistical analysis and on tools of computational mass spectrometry to elucidate the input and fate of contaminants in surface water. The software tool MetFrag has been used to retrieve chemical structures of PubChem and to match the measured with in silico fragmentation patterns. A variety of compounds from pesticides, PPCPs and their metabolites could be found. The number of compounds showed an increasing trend from the source of the creek Ammer to the mouth which could be attributed to mainly to the input of wastewater treatment plant effluents. In the source region the herbicide metabolite desethyl atrazine indicates a historical background of atrazine pollution. Further intermittent findings of amidotrizoic acid in the source region could be traced back to the influence of storm water overflow. Samples taken downstream of a wastewater treatment plant showed more then 10 000 mass peaks retrieved from the chromatogram by a deconvolution software. Typical wastewater indicators have been found like the X-ray contrast media amidotrizoic acid, iomeprol and iopromide, or further pharmaceuticls like carbamazepine and diclofenac, but also the artificial sweeteners acesulfame and sucralose. Further candidates were retrieved from the huge mass list by PCA, e.g. to select relevant candidates for wastewater input. Several compounds could be identified by the application of PubChem search and mass fragmentation match using MetFrag. However, there is still a large number of candidates to be identified due to inconsistent data base and fragment match and due to a high number of possible isomers. In conclusion, there remains a more urgent call for the availabililty of mass spectral libraries with sufficient numbers of entries.

EP05-6
Can bioanalytical tools help us ensure that our water is safe?
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Chemical monitoring provides a quantitative assessment of single organic contaminants in a water sample but cannot account for the presence of non-target compounds such as unidentified transformation products and interactions between chemicals. Bioanalytical monitoring is complementary to chemical analysis and provides information on all bioactive micropollutants in a sample according to potency, i.e., chemicals of higher toxicity will be weighted higher than less toxic chemicals. Cell-based bioassays provide measures of the cumulative effects of chemicals that exhibit the same mode of toxic action, for which the selected bioassays are indicative, and they can give a measure of the cytotoxicity of all chemicals acting together in a water sample. Improved detection of the presence of chemicals in water enhances risk assessment and informs water management options, among them water recycling from impaired sources such as sewage, or stormwater harvesting and reuse. In this presentation the design of a modular battery of bioassays based on toxicological principles will be presented.. This bioanalytical test battery was used for monitoring organic micropollutants across an indirect potable reuse scheme testing sites encompassing the complete water cycle from sewage to drinking water to assess the efficacy of different treatment barriers, including source control, wastewater treatment plant, microfiltration, reverse osmosis, advanced oxidation, natural environment in a reservoir and drinking water treatment plant. The results of the various studies presented here indicate that bioanalytical tools provide valuable additional information to chemical analysis and should be implemented in the future as a monitoring tool.

EP06 - Perfluorinated compounds: From emission sources to the place of impact

EP06-1
Estimating emission source strength of four poly- and perfluorinated alkyl substances (PFASs) in Zurich, Switzerland, using a measurement-and-modelling-combined technique
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Recent studies show that urban areas can be important sources of many semi-volatile organic chemicals (SVOCs), including poly- and perfluorinated alkyl substances (PFASs, e.g. fluorotelomer alcohols (FTOHs) and perfluorooctane sulfonamides (FOSAs)). These PFASs are released in urban areas during use and disposal of polymeric materials, chemicals or consumer products that contain PFAS residuals and directly from manufacturing sites. However, residuals vary considerably among consumer products and it is therefore difficult to estimate diffusive emissions based only on surveys of a limited number of consumer products. In this work, we address this problem by using a multimedia mass balance model to interpret measurements of four PFASs (8:2 FTOH, 10:2 FTOH, Me-FOSA and Et-FOSA) made in the city of Zurich (Switzerland), during a sampling campaign performed in August 2010. The model has been designed to quantitatively capture the day-night cycling of SVOCs in air considering the atmospheric boundary layer dynamics. The estimated yearly average emission source strength of the four PFASs are in the range of 0.4 to 22.5 kg/year and follow the sequence: 8:2 FTOH > 10:2 FTOH > Me-FOSA > Et-FOSA. To obtain more insight into the emission pathways of the four PFASs, the emission strengths estimated from our study were compared to literature data. In general, our estimated emission source strengths of FTOHs are in good agreement with other estimates, which are all based on emission factors along the life cycle of FTOHs. Moreover, our estimates confirm that there is still low but ongoing volatilization of MeFOSA and EtFOSA from consumer products manufactured prior to 2002, when the major producer stopped producing these substances. It may still take years until they disappear from consumer products and the environment. Note, there is no PFAS-related industry in Zurich, therefore, our study shows that diffusive emissions during the use and disposal phase alone are noticeable sources of these PFASs to the environment, in addition to the direct emissions from manufacturing sites.

EP06-2

Percolation characteristics of perfluorinated compounds (PFC) in soil and carry over in plants as shown by long-term lysimeter studies

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Perfluorinated compounds (PFC) can be taken up by crop plants from the soil of contaminated agricultural lands. Soil contamination may take place through irrigation with PFC contami-nated water or by fertilization with sewage sludge containing PFC (1,2).
Precipitation, e.g. rainfall may result in only a proportion of the PFC reaching the plants since highly water soluble PFC may be washed out of the soil. Discharge from a landfill containing PFC may also lead to contamination of the food chain via percolation water. A lysimeter study is therefore particularly well suited to observe the carry over in plants and also any pos-sible displacement of the substances from the soil to percolating water. In addition, a long-term study provides information on the time-dependant uptake and leaching of PFC.
Lysimeter studies were carried out on monolithic soil columns (with a volume of 1.5m3). The lysimeter soil received a single treatment with an aqueous solution of a technical mixture of PFOA and PFOS at a concentration of 25mg/kg soil. Over a period of 5 years the harvests of wheat, rye and rape (canola) were tested for the presence of PFC. Grain and straw were meas-ured separately. Once per month the percolation water was also tested for PFC concentration. In addition to PFOA and PFOS, PFBS, PFPeA, PFHxA and PFHpA, as impurities in technical PFOA or PFOS, were all detected in both the plant material and in the percolation water. The concentrations of PFBS (an impurity of PFOS) were as high in plant material as those of PFOA and PFOS in the first year of testing (2007). This indicates that short-chain PFC are taken up by plants much more quickly than those of longer chain lengths. In addition, PFBS, PFPeA, PFHxA, PFHpA and PFOA enter the soil more rapidly than PFOS, the latter only becoming measurable in the percolation water after 4 years. Preliminary results from the long-term studies will be presented.
(1) Stahl T, Heyn J, Thiele H, Hüther J, Failing K, Georgii S, Brunn H (2009) Carry Over of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) from Soil to Plants. Arch Environ Contam Toxicol 57(2):289-298
(2) Lechner M, Knapp H (2011) Carryover of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) from Soil to Plant and Distribution to the Different Plant Compartments Studied in Cultures of Carrots (Daucus carota ssp. Sativus), Potatoes (Solanum tuberosum), and Cucumbers (Cucumis Sativus). J Agric Food Chem 59(20):11011-11018

EP06-3

Uptake of Perfluoroalkyl acids by hydroponically and field grown Lettuce (Lactuca sativa) and Radish (Raphanus sativus)

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Perfluorinated alkyl acids (PFAAs) are bioaccumulative persistent, organic pollutants (POPs), which can be detected ubiquitously in the environment. PFAAs pose a risk to human health due to accumulation in the food chain. The occurrence of PFAAs in animals, such as fish, birds and mammals including humans is fairly well documented, but little can be found in the literature about crops or plants in general. Also, most studies focus just on the two main compounds perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA).
Humans are possibly exposed to PFAAs through consumption of vegetables and other plant-related food items. The objective of this study is to understand the accumulation process of PFAAs in crops.
In a greenhouse experiment lettuce (Lactuca sativa, var. attraction) was grown hydroponically with a spiked nutrient solution to avoid sorption to soil and to make sure the offered PFAAs are completely bioavailable. The lettuces were exposed to a set of 10 perfluorinated carboxylic acids (PFCAs) and 3 perfluorinated sulphonates (PFSAs) in four different concentrations to assess the difference in behavior between PFAAs and concentration dependencies.
In a field experiment lettuce and radish (Raphanus sativus) were grown in lysimeters in 4 different concentrations of spiked soil to have a comparison to the greenhouse experiment and a comparison of a leafy vegetable to a root/bulb vegetable.
The results of the concentrations in the different parts of the plants show a different pattern than in the greenhouse experiment with higher concentrations in the foliage part for most of the compounds.

EP06-4

Perfluoroalkyl substances in raw and processed vegetables and fruits collected in four European countries; PERFOOD

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The human diet is considered a general source contributing to the overall PFAS burden of the human population. Possible exposure pathways include beverages, food in general and migration from food packing or cookware 7,8. In the EU project PERFOOD, standardized selection of food items, sampling procedures and analytical methods as well as evaluation strategies were applied, enabling a unique assessment of the occurrence of PFASs in European food as well as the identification of major sources of PFAS exposure via food. During the sampling campaign more than 800 raw food items were purchased, homogenized and after pooling analysed in selected laboratories. This presentation will cover the analytical results for vegetables and fruits acquired in Norway, Belgium, Czech Republic and Italy in perspective to other food items consumed regularly.
In general the PFAS levels found were very low, and mostly short chained PFASs up to C8-chains were detected. In general, the most PFASs were detected in samples from Belgium and Norway, followed by samples from Italy and Czech Republic. Mainly PFCAs were detected and occasionally some PFSs, in few samples.
In general, vegetables and fruit seem not to be a main contributor to the human exposure of PFASs via food if not harvested close to point sources. However, data from Belgium show that plants consumed as vegetables and fruit in human diet are able to take up a number of PFASs in the edible parts when exposed to them. Results for the processed vegetables and fruit items will be presented in the presentation.
From the investigated vegetables, spinach, lettuce, asparagus, fennel and potatoes, showed highest content of PFAS and will be followed up in the second sampling campaign. Courgettes, cucumbers, aubergines, and mushrooms showed lowest levels of PFAS. After grouping the single vegetable species we found the following order of decreasing PFAS content: Leafy vegetables > potatoes > pulses & legumes > stem vegetables.

EP06-5

Perfluoroalkyl acids in blood serum from first time mothers from Uppsala, Sweden: temporal trends 1996-2010 and serial samples during pregnancy and nursing

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Concentrations of 13 perfluoroalkyl acids (PFAAs) were determined between 1996 and 2010 in pooled blood serum samples from nursing primiparous women living in Uppsala County, Sweden. The aim was to investigate possible effects on human exposure following risk management measures to reduce production, emissions and use of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). PFOS levels declined on average 9% per year from over 20 ng/g serum in 1996 to below 10 ng/g

serum in 2010. PFOA levels declined with 3% per year, from around 3 ng/g serum to about 2 ng/g serum. Thus, certain sources of human exposure to these two compounds have been eliminated. On the other hand, levels of perfluorinated sulfonic acids with 4 (PFBS) and 6 (PFHxS) carbons increased on average 14 and 8.3% per year, respectively. To our best knowledge, this is the first report of statistically significant upward trends for PFBS and PFHxS in human serum after the year 2000. In 2010 the serum levels of PFHxS and PFOS were comparable. Levels of perfluoroalkyl carboxylic acids with 9 (PFNA) and 10 (PFDA) carbons increased 4.6 and 3.8% per year, respectively. Consequently, exposure to PFBS, PFHxS, PFNA and PFDA and/or to their precursor compounds has increased after the phase-out of the C8-chemistry by the 3M Company in 2002. Moreover, serial maternal serum samples during pregnancy and nursing as well as corresponding cord blood were analysed to evaluate if PFAA levels during the nursing period are representative for the fetal development period. PFOS, PFOA and PFNA levels decreased during pregnancy. PFNA declined twice as much as PFOA, suggesting compound specific differences in the blood dynamics. Strong correlations were found between PFAA levels during pregnancy, the nursing period and the cord blood. Thus, maternal PFAA levels determined after delivery can be used as a good estimate of fetal exposure. Mean serum/whole blood concentration ratios of PFOA, PFNA and PFOS were also determined and found to be close to 2, reflecting a volume displacement by red blood cells in whole blood. However, large inter-individual differences were observed with some individuals displaying a ratio close to 1, suggesting a distribution of PFAAs also to the blood cellular fraction.

EP06-6
Does exposure to 8:2 FTOH affect lung function?
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Early life exposures to perfluorinated chemicals (PFCs) have been shown to alter lung and immune function in animal models. We exposed pregnant BALBc dams (GD16) to 100mg of 8:2 FTOH that was coated onto a cardboard enrichment huts which were replaced weekly. Dams continued to be exposed to FTOH in this way and pups were born in the same environment. Upon weaning, 4 female pups were placed into each cage where they continued to be exposed to FTOH is the same way. Control dams and pups received untreated cardboard enrichment huts. One half of the females of each litter received ovalbumin (OVA) IP at days -21 and -4 and intranasal OVA day -4, -3 and -2. Airway resistance (Raw) was measured by flexiVent at 10 weeks of age. Methacholine (MCh) was nebulized and administered in incremental concentrations from 3-50 mg/ml. PC200, the concentration of MCh required to increase the airway resistance 3X from baseline airway resistance was calculated. The PC200 for FTOH exposed animals was significantly less compared to controls. This suggests that early life exposures to 8:2 FTOH may be playing a role in increasing airway hyperresponsiveness in the human population.

EP07 - Plastics: an emerging risk to the marine environment

EP07A-1
Microplastics in the marine environment: synthesis and next steps
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Marine debris is a global problem along coastlines as well as urban, coastal, and offshore waters. Plastic marine debris has been found in surface waters since the 1970s, and an increasing number of observations report plastic debris in the most remote areas of the global oceans. This global long-range transport is possible due to the shared physical properties of many plastic polymers which make them resistant to degradation in the environment. Weathering can slowly break large plastic products into pieces, called microplastics, but full mineralization takes much longer and microplastic particles are expected to persist in the environment for decades. Among many potential risks to the marine environment, microplastics can physically block gastrointestinal tracts of organisms that accidentally ingest these particles, and can serve as vectors of chemicals into marine organisms and environments. This presentation will synthesise known environmental concentrations of microplastics using both historical reports and in-depth, original data from the Chesapeake Bay, USA; present the current state of the science regarding the impacts of microplastics; and discuss future steps for this emerging field of research.

EP07A-2
UK marine litter monitoring
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The distribution of marine litter on the UK seafloor, including different types and quantities, were mapped by collecting additional data on the back of existing research cruises. Several surveys using specific types of trawls gathered detailed information over a total time period of 19 years (1992 until 2011). The fieldwork experiences allowed us to develop a standard sampling procedure with easy to use datasheets, which facilitates further analysis and future harmonisation across surveys worldwide. The results suggest widespread distribution of marine litter on the seabed of the North Sea, dominated by plastics. The data shows detailed distribution and accumulation patterns in North European waters. There is a considerable variation in geographical abundance between stations, ranging from 0 to 3224 items of debris per km2. Plastic (mainly bags and bottles, 30%) accounted for a very high percentage, more than 70%. Remarkably, the available trend data indicates that quantities of macro marine litter remained relatively stable over the past two decades. In order to investigate this further, the benthic surveys were reinforced with a one off UK case study on marine litter in the water column (2011). High quantities of microplastics were found near major river outlets (>100000 particles/km2). The results will be used to inform policy makers when designing programmes of measures. This analysis is a first valuable contribution to assess marine litter in North European waters and may eventually be used to determine Good Environmental Status (GES) as defined in the EU Marine Strategy Framework Directive, Descriptor 10.

EP07A-3
Microplastics in the North Sea region: what kind of quantities and associated ecological effects are we looking at?
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The information available on the composition and distribution of microplastics (<5 mm) in the North Sea region is rather scarce because the majority of surveys to date have focused on macro-sized plastic litter. Microplastic effects studies are still in their infancy. While society broadly agrees that plastic simply doesn't belong in the marine environment, regulators are understandably calling for answers to the question, what kind of damage are microplastics doing? Incipient studies dedicated to marine microplastics effects need more time to produce a robust body of results. Therefore in this paper, we combine the current scientific knowledge about the amounts and distribution of microplastics in the North Sea region sediments, water and biota with particle toxicity knowledge of these materials from other fields of study in order to estimate the risk of microplastics in the North Sea to the individual organisms and humans exposed to them. By combining data from diverse fields of study (oceanography, ecotoxicology, drug delivery science, marine biology, etc.) we conclude that field and laboratory evidence for internal exposure in biota gives us an early warning that microplastics are likely to be regarded as bioaccumulative materials and prime biofilm substrates. Considering nano and microparticle toxicity and drug delivery data, we deduce that a variety of effects can be expected depending on the size category of the microplastics in question, since the propensity of particles to be sorbed or eliminated via various routes by various tissues is inherently size-dependent. Inflammatory responses (immune system), cellular damage and other possible physiological, ecological or behavioural responses can be expected after the introduction of foreign materials into biological tissues via the gastrointestinal or respiratory systems etc. The bouquet of evidence presented in this paper supports the position that synthetic plastic polymers are fundamentally incompatible materials for input into biological cycles.

EP07A-4
Plastic loads on coastal shorelines: where do the micro-plastics come from?
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Plastics have been entering the world oceans for over half a century. Beaches and shorelines are commonly sinks for such debris. However, despite growing concerns over debris impacts and increasing actions to remedy these problems, an understanding of the role micro-plastics play in this and the potential impacts these micro-plastics are having, is still in its infancy. With this in mind coastal and harbour beaches with different known macro-plastic loads were sampled within Central Queensland, Australia to determine the proportion of debris in different size categories.
Debris was collected using strandline transects and randomised quadrats (0.5m2). All surface debris was collected down to a depth of 5cm. Samples were then sieved into different size classes and further categorised (eg. Pellet, fragment).
Results indicate that areas with high macro-plastic loads also have high micro-plastic loads. Conversely, low impact sites had proportionately less micro-plastics than the corresponding macro-plastic loads. The nature of the shoreline(eg. Degree of wind and wave energy and current patterns) appear to influence the composition of plastics on these shorelines with high energy environments contributing to the localised breakdown of debris rather than these micro-plastics originating from outside the local area. Further data trends will be presented and the potential impacts to biota and implications for management discussed.

EP07A-5
Sorption and desorption of persistent organic pollutants from microplastics in the marine environment

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Microplastics are small fragments of marine debris. Such fragments now appear to be widespread in the marine environment and have been reported at the sea surface, on shorelines and on the sea bed. Microplastics have been defined as particles less than 5 mm in diameter, but fragments much smaller (< 20 microns) than this are widely reported including pieces of nylon, polystyrene, polyethylene and PVC. It has been suggested that microplastics present potential mechanisms for the transport of persistent organic pollutants (POPs) and the release of chemical additives from plastics, to organisms. Particles of unplasticised PVC (uPVC) and ultra high molecular weight polyethylene (UHMW PE), in the size range 200 to 250 µm diameter, were investigated for their potential to sorb and desorb 4,4'-DDT and phenanthrene in seawater and using sodium taurocholate in order to simulate conditions in the gut of marine organisms. Sorption capacity and binding strength was found to be pollutant and polymer specific. Equilibrium distribution coefficients (K_d) for the sorption of phenanthrene onto PE were higher than for PVC while the K_d values for the sorption of DDT were higher for PVC than PE. The desorption of phenanthrene and DDT from plastic was faster in sodium taurocholate than in seawater, but is slower than desorption of organic contaminants from natural sediments. The results suggest that DDT and phenanthrene will desorb from plastics faster as a consequence of digestion by marine organisms than in seawater alone.

EP07A-6
Simultaneous analysis of microplastics and associated plastic additives in sediments
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Microplastics of a size of 5 mm and less in length accumulate in sedimentary marine habitats. They contain organic additives, which could have possibly toxic or endocrine effects when ingested by marine organisms. A universally applicable method was developed to analyse microplastics and associated organic plastic additives (OPAs) in sediments simultaneously.
Six samples of dry sediments with a mass of 1 kg were spiked each with 10 pieces of polypropylene (PP), polyethylene (PE), polycarbonate (PC), polyvinylchloride (PVC), polyurethane (PU), polyamide (PA), polyethylene terephthalate (PET) and acrylonitrile butadiene styrene (ABS). The sizes of the synthetic polymer pieces were about 1 mm in length. Microplastics were sorted out using an air-induced-overflow method and a sodium chloride (NaCl) solution. After this procedure, weights between 50 and 150g were left. Subsequently the polymers were separated from the sediments by flotation in a glass volumetric flask using a sodium iodide solution (NaI) (60%, w/w) which enabled the separation of polymers with a density < 1.804 g/cm³ at 20°C. The flask was closed with a glass plug and shaken for 30 s. After 15 min the surface of the NaI solution was carefully decanted into a glass beaker while decantation of any sediment was strictly avoided. This procedure was repeated five times. The solution was filtered using a vacuum filtration unit equipped with a 0.45 µm cellulose acetate-nitrate filter (Mili, China). Then the filter was washed with H2O2 (30% v/v) and the filter residue was stored in the H2O2 for one week to remove the biogenic organic material. The solution was filtered again using a glass fibre filter (Whatman, USA) with a pore size 2.7 µm. Recoveries of the whole procedure were between 70 and 100 %.
Polymer particles were analyzed for OPAs and polymer types by thermal desorption-gaschromatography (TD-GC) and pyrolysis-GC (Pyr-GC) in independent runs. The GC was equipped with a cooled injection system (CIS). OPAs and pyrolysis products were identified running a mass spectrometer (MS) in selected ion mode and full scan mode, respectively. Several organic polymer additives e.g. polycyclic aromatic hydrocarbons were identified. For all polymers typical pyrograms were obtained.

EP07B-1
Leaching of halogen free and brominated flame retardants from plastics under different conditions
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Information about the production, distribution and consumption of flame retardants in electric and electronic (E&E) equipment (plastics) is well described. However, there is a knowledge gap in the amount of flame retardants leaching from plastics of electric and electronic (E&E) equipment to the environment. In the EU funded project ENFIRO, halogen free flame retardants (HFFR) are studied that are viable alternatives to specific commercial brominated flame retardants (BFR). Leaching studies of BFRs from different types of plastics have been described in literature; however, limited information on leaching of HFFRs is available. ENFIRO studies 15 HFFRs of which 6 are metal-based. Metal-based flame retardants are stable in plastic (polymer) products, but can leach, dissociate and enter the environment. Monitoring of the fate of metal-based HFFRs in the environment is difficult as metals can have various sources of emission. Leaching tests of HFFRs from plastics is an alternative method that may contribute to the exposure and risks assessment and understanding of the fate of HFFRs in the environment. The current study shows leaching properties of different HFFRs from polymers in comparison with BFRs. Thereby, the influences of pellets vs. moulded plates and pH on the leaching properties are studied. Two types of leaching protocols were tested. The TLCP protocol, from the US EPA, use worse-case leaching conditions (low pH) to simulate a municipal waste landfill, and studies if waste has toxic characteristics and is hazardous. The second protocol (DIN 38414-S4) determines leaching by water (neutral pH), and has been widely used for regulatory purposes in Europe. We show that no differences in leaching properties between the DIN and TCLP methods for two metal-based PBT pellets were found. However, higher leaching rate coefficients of HFFRs from PBT pellets than PBT moulded plates were found, which is probably a results of the differences in surface:volume ratio and the porosity of the materials. Also the influences of nanoclay on the leaching behaviour of the HFFRs have been studied. Additional experiments were performed with marine and freshwater to study the influence of leaching conditions (salinity, humic acids, pH) to simulate different environmental conditions.

EP07B-2
Use of immunofluorescence technique in cultured fibroblasts from cetaceans as new 'in vitro' tool to investigate effects of microplastic
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Each year in the world more than 240 million tonnes of plastic are used and discarded 'end-of-life' plastics accumulate particularly in marine habitats, where contamination stretches from shorelines to the open-ocean and deep-sea. Degradation into smaller pieces means particles <5 mm, usually defined as microplastic, which are now considered a new priority in marine environment contamination. In fact, ingestion of microplastics, that can be taken up and stored by tissues and cells, provides a potential pathway for the transfer of hydrophobic organic contaminants, monomers, and plastic-additives to organisms with uncertain consequences for their health. Contaminants such as phthalates, bisphenol A (BPA) and polycyclic aromatic hydrocarbons (PAHs) are some of the principal constituents of plastic. The aim of the present study is to propose immunofluorescence technique in cetaceans cultured fibroblasts as a new "in vitro"tool to explore the susceptibility of these marine mammals to different pollutants related to marine microplastic contamination. The cell lines were cultured from biopsies of free-ranging cetaceans and skin tissue from stranded cetaceans (dead within 2-12 h). Using an indirect immunofluorescence assay, we detected endogenous proteins induced by different contaminants. Here we present the method used for qualitative and quantitative evaluation of two isoforms of Cytochrome P450 (CYP1A1 and CYP2B) induced by BPA and PAHs. Cells were treated for 48 h with contaminants in sterile culture plates with wells having individual sterile covers. After fixing and extraction with methanol and acetone at -20°C, we conducted a first reaction with primary antibodies: anti rabbit cytochrome P450 1A1/1A2 and anti 2B4 cytochrome P-450 diluted 1:500 for 1A1/1A2 and 1:100 for 2B4, for 2 h. Cells were then treated with the respective secondary antibodies (goat anti-rabbit) labelled with a fluorochrome, diluted 1:400, in the dark. Fluorochrome was detected using a solution containing 40% CITIFLUOR and 60% PBS, whereas DAPI was used as marker of chromatin for cell count. The reaction was read using a fluorescence microscope (Olympus mod. BX41). Immunofluorescence was quantified with a specially designed Olympus macro, *DetectIntZ*. A major result was the possibility of using this "*in vitro*"assay to evaluate the susceptibility of different cetacean species to microplastic components.

EP07B-3
Relative bioavailability of PCBs associated with microplastic
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Microplastics are present in shoreline sediments worldwide, are known to accumulate hydrophobic organic compounds like polychlorinated biphenyls (PCBs) from water and many different organisms have been observed to ingest small plastic particles; therefore, plastic debris may pose a potential harm to aquatic ecosystems from the transport and exposure of toxic chemicals. The objective of this work was to measure the bioavailability of microplastic-associated PCBs to benthic invertebrates in comparison to other environmentally-relevant particle types (all <63 µm diameter) in two ways: bioaccumulation in an oligochaete worm L. variegatus in 7-d exposure tests of sediments amended with PCB-laden particles, and digestive solubilization of contaminants from particles in a gut fluid mimic of the polychaete A. marina. The percent solubilization of PCBs by gut fluid was greatest for wood, followed by biochar, polypropylene (PP), and then coal. PCBs associated with PP were expected to be more bioavailable than the black carbons, coal and biochar, based on the lower solid-water distribution coefficients for PP. The observation of relatively lower digestive bioavailability from PP than biochar may indicate that solubilization was kinetically limited by diffusion from the interior of the plastic. Kinetic release of contaminants depends in part on the surface area to volume ratio of particles, which was very low for PP compared to the black carbons. In contrast to gut fluid solubilization, results of the bioaccumulation tests indicate that PCBs associated with PP are more bioavailable than with biochar. Total PCB concentrations in lipid measured in worms exposed to sediments with loaded PP and biochar were 76% and 97% less than spiked sediment, respectively. In these 7 d exposures, PCBs associated with plastic had more time to repartition with pore water and worm

lipids than the gut fluids in the 4 hour in vitro digestion. At the PCB homolog level, biouptake of less-chlorinated PCBs was greater for exposures with PP, indicating release to sediment pore water followed by dermal contact may be an important route of exposure. Thus, plastic debris in the environment appears unlikely to contribute significantly to digestive uptake of PCBs to organisms, that is perhaps, until weathered plastic particles are small enough so that kinetic release during the time-frame of digestion is not limiting, or if plastic particles are retained in the digestive tract.

EP07B-4

Occurence of microplastics in mussels (Mytilus edulis) and lugworms (Arenicola marina) collected along the Belgian-French-Dutch coast

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Laboratory experiments have shown that various marine organisms ingest microscopic particulates. Microplastics smaller than 10 µm were even shown to translocate from the gut to the circulatory system of mussels. However, in all these experiments the exposure concentrations (range 1000 - 50,000 mg.kg-1) were much higher than any field concentration (range 50 - 200 mg.kg-1). As such it is difficult to assess the relevance (risks of adverse effects) of these laboratory observations for organisms living in natural marine environments. The aim of this project was to study the presence, and if present, the concentrations of microplastics in two marine species collected in the field: (1) the blue mussel Mytilus edulis and (2) the lugworm Arenicola marina.
Biota (mussels and lugworms), seawater and sediment from the intertidal zone were collected at 6 beaches along the French-Belgian-Dutch coastline, during September-October 2011. Microplastiscs were extracted from the sediment using elutriation followed by a sodium iodide (NaI) extraction, in order to separate the lighter plastic particles from the heavier particles. Seawater was filtered and a NaI extraction was performed on the settled particles Organisms underwent an acid (HNO3) digestion procedure. Quantification of the extracted particulates showed that - at all sampling locations - all tissue and faeces samples contained these particulates. Tissue concentrations for mussels was around 2 particles.gram-1 of tissue. Lugworms contained only slightly higher concentrations of particulates: 3 particles.gram-1 of tissue. As expected, these tissue concentrations are low compared to the concentrations present in the environment: water contained about 80 particles.L-1 and sediment 40 particles.kg-1 sediment. Although the units are clearly different, these data can be used to illustrate the difference between the environmental and tissue concentrations. Sediment also seemed to have a lower concentration of particulates than seawater. This can be explained by the fact that the sampled upper 5 cm of the intertidal zone is a very dynamic system with high perturbation levels, making it difficult for the suspended particles to settle.
The use of these organisms allowed, for the first time, the assessment of the transfer of microplastics from both the water as well as the sediment matrix to marine life.

EP07B-5

Detection of polybrominated diphenyl ethers (PBDEs) in tissue of seabirds ingesting plastics

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Polybrominated diphenyl ethers (PBDEs) were measured in the abdominal adipose tissue of short-tailed shearwaters, Puffinus tenuirostris, that were accidentally caught during experimental fishing in the North Pacific Ocean. Their ingestion of plastics have been frequently observed. All the examined seabirds (i.e., 7 individuals) contained plastics in their stomachs with 0.04 g to 0.41 g per individual. PBDEs were detected from all the seabird samples with concentration range of total PBDEs from 0.5 to 5.4 ng/g-wet tissue. In one individual, BDE#209 was significantly detected. BDE#209 was normally not detected in marine fish and, therefore, exposure of BDE#209 to seabird through food web is unlikely. On the other hand, BDE#209 was detected in marine plastics. The detection of BDE#209 in the seabird may be ascribed to transfer of BDE#209 from ingested plastics to the seabird tissue. In addition to this individual, two individuals showed sporadic higher concentrations of PBDEs in their tissue. They were dominated by BDE#153 and BDE#154 and their congener profiles were different from the other individuals. No significant correlation was observed between tissue PBDE concentrations and the amounts of plastics in their stomachs. The large variabilities in tissue PBDE concentrations may be explained by large variability in PBDE concentrations in plastic fragments. Our measurement of PBDEs in marine plastics showed large fragment-to-fragment variation among the plastics collected from the same sites. These variabilities can be explained by heterogeneity of plastic fragments in terms of contents of additives and residence time in seawater. Heterogenic nature of the ingested fragments may explain the variation of PBDE concentrations among the seabirds and no significant correlation between tissue PBDE concentrations and plastic ingestion. Analyses of PBDEs in ingested plastics and natural prey are necessary. More number of the seabirds with different amounts of ingested plastics, including individuals with no plastic ingestion, should be analyzed to further examine the transfer of PBDEs from ingested plastics to seabird tissue.

EP07B-6

Preliminary results on the potential assumption of microplastics by Mediterranean Fin whale: the use of phthalates as a tracer

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Micro debris floating on the Mediterranean Sea have reached 115,000 particles per km2 with a maximum of 892,000 particles. Impacts of microplastics on organisms and the environment are largely unknown. More than 180 species have been documented to absorb plastic debris including planktophagous species. Until now no data are reported on the potential assumption and effects of microplastics on baleen whales. In this paper we explore for the first time the assumption and potential impact of microplastics in the mysticete species Fin whale (Balaenoptera physalus), suggesting the use of phthalates as a potential tracer of microplastics assumption by Fin whale through micro litter and plankton ingestion. The Fin whale, the only resident mysticete in the Mediterranean Sea (concentrated during the summer in the Marine Protected Area (MPA) Pelagos Sanctuary), feeds largely on the planktonic euphasiacean species, with each mouthful can trap about 70,000 liters of water (including the surface feeding activities), could potentially undergo to the potential risk of the ingestion and degradation of microplastics. The project is implemented through three main steps: Phase I - collection and count of microplastics in superficial plankton samples in Pelagos Sanctuary; Phase II - ecotoxicological investigation of phthalate content in superficial plankton samples of Pelagos Sanctuary; Phase III - ecotoxicological investigation of phthalate content in stranded Fin whale specimens collected on the Italian coasts. Among the 23 superficial plankton samples, 13 have shown the presence of plastic particles. The highest "microplastic density"(9.67 debris/m3) was found in the sample collected close to the Portofino MPA (Ligurian Sea). High concentration of phthalate MEHP and DEHP have been detected in superficial plankton samples collected in the Pelagos Sanctuary areas, with values approximately four time higher in the samples of the Ligurian Sea than the samples of Sardinian Sea. Regarding chemical harm to Mediterranean Fin whales, related to the potential assumption of plastic derivates, the preliminary data of this paper underline for the first time the presence in the blubber of four stranded Fin whales relevant concentration of MEHP. This data suggest the use of phthalates as a potential tracer of microplastics assumption by Fin whale by micro litter and plankton ingestion.

EP08 - What is the current state of the science on the fate, exposure and effects of pharmaceuticals in the environment?

EP08A-1

Environmental progestin concentrations disrupt oogenesis in amphibians

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Progestins (synthetic progesterone) are extensively used in human and veterinary medicine in e.g. contraceptives and in other hormonal therapies. Resent research shows that progestins pose a threat to egg laying in wild fish. Information on the susceptibility of frogs to impacts from environmental progestin concentrations is lacking. The present study aimed to 1) characterize progestagenic effects on the full cycle of oogenesis (egg development) in frogs, and 2) determine female amphibians' susceptibility to reproductive impacts from an environmental progestin. Levonorgestrel is a commonly used progestin found in sewage treatment plant effluents at concentrations up to 30 ng/L. Sexually mature female Xenopus tropicalis were exposed to levonorgestrel via the surrounding water for 28 days (0, 1.3, 18, 160 or 1240 ng/L). Their ovaries were analyzed histologically with respect to frequencies of immature (in early meiotic prophase I), previtellogenic, vitellogenic, mature, and atretic oocytes. Levonorgestrel exposure caused reduced proportions of oocytes at immature, vitellogenic and mature stages and increased proportions of previtellogenic oocytes in the ovaries, compared with the controls. The lowest tested concentration,1.3 ng/L, increased the proportions of previtellogenic oocytes and reduced the proportions of vitellogenic oocytes, indicating inhibited vitellogenesis. The present study shows that progestin concentrations found in the aquatic environment impaired oogenesis in adult frogs. Our results indicate that progestagenic effects on oocyte development include interrupted germ cell progression into meiosis and inhibited vitellogenesis. Considering the crucial role of oogenesis in female fertility our results indicate that progestins in the environment may pose a threat to reproduction in wild amphibian populations at contaminated sites.

EP08A-2

Identification of active synthetic steroid compounds in impacted river downstream from pharmaceutical industry

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The environmental occurrence of emerging pollutants able to disrupt endocrine signalling pathways other than those mediated by estrogen receptors (e.g. corticosteroids, androgens or progestagens) is of recent concern. Recent evidences have suggested that effluents from pharmaceutical industry release drugs into rivers and trigger adverse effect on wildlife. By using in vitro bioassays combined to passive sampling (i.e. Polar organic compound integrative sampler, POCIS), we previously reported the occurrence of estrogenic, glucorticoidic, anti-mineralocorticoidic, progestative compounds and pregnane X receptor (PXR) ligands downstream a pharmaceutical industry release where strong reproductive alteration have been reported in fish. In this study, we report the use of effect directed approach to identify the compounds responsible for these

activities. We first assessed mass balance calculation through chemical analyses directed by toxicity profile. Chemical analyses in POCIS crude extract showed the occurrence of high concentrations of dexamethasone, spironolactone, 6-methyl-prednisolone (up to 100 µg/g of sorbent) that well explained GR and anti-MR activities (up to 100%). Conversely, other biological activities (i.e. estrogenic, PXR-like) were poorly explained by the detected chemicals. Then, sample RP-HPLC fractionation was carried out to unravel the different activities. It allowed the isolation of estrogenic and PXR-like activities from GR, PR and anti-MR. It also revealed the occurrence of MR agonists that were masked by the strong anti-MR activity in the crude extract. In addition, HPLC calibration showed a good fitting between retention times of detected chemicals and several active fractions (e.g. 6-methyl-prednisolone in F11, dexamethasone in F12). Nevertheless, some active fractions were left unexplained by calibration standards suggesting that many active chemicals remain to be identified. Chemical analyses in these fractions are under investigations using LC-HRMS system and results will be presented. Our study demonstrates (1) the usefulness of MBA approach using pre-directed chemical analyses based on toxicity profile (2) the strength of the fractionation to unravel complex mixture effect and to finely isolate active chemicals. Overall, our results underscore the need to increase knowledge on the effects of corticosteroids and progestogens on aquatic organisms for better risk assessment.

EP08A-3

Mechanism of action of human pharmaceuticals in fish: the 5alpha-reductase inhibitor dutasteride as case study

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In recent years, a growing number of human pharmaceuticals have been detected in the aquatic environment, generally at low concentrations (sub-ng/L to low µg/L). These compounds are characterised by highly specific mechanisms of action (MoA), high potency, and prolonged activity in order to minimise dosing requirements and potential toxicity in patients. The functional and evolutionary degree of conservation of the drug target in non-target species, together with a cross-species extrapolation of pharmacological and toxicological information generated in mammals during the drug development phase should be considered as key factors in the environmental risk assessment process. Our research addressed the question of whether or not dutasteride, a human pharmaceutical mainly used to treat benign prostatic hyperplasia, may cause adverse effects in the teleost fathead minnow (*Pimephales promelas*) by inhibiting the activity of both isoforms of 5α-reductase (5αR), the enzyme that converts testosterone into dihydrotestosterone (DHT), despite the general assumption, in fish endocrinology, that DHT is not synthesized in teleosts, or if it is, it has modest or no physiological relevance. To our knowledge, this class of pharmaceuticals (5αR-inhibitors) has never before been tested on any fish, or any other aquatic species. The experimental work presented here was divided into two phases: Phase I, focused on the target, and Phase II focused on the effects of the drug in fish. The results of the Phase I showed that 5αRs are evolutionary conserved in the fathead minnow, both 5αR1 and 5αR2 genes were expressed in the testis, and DHT was detected in fish plasma at concentrations comparable to the human ones. These results strongly suggested that DHT has a physiological role in the fathead minnow, and constituted the rationale for testing the effects of dutasteride in this species. Dutasteride caused significant adverse effects in all the in vivo studies performed during the Phase II in order to evaluate its potential toxicity on fish, including early life stage and short term reproduction studies, and all the tested life stages were sensitive to the inhibition of 5αRs activity; however, none of the observed adverse effects occurred at concentrations of exposure lower than 32 µg/L indicating that, at present, the potential presence of dutasteride in the environment (PEC=0.03 ng/L) does not represent a risk to wild fish populations.

EP08A-4

Sub-lethal effects induced by the main cocaine metabolite, the benzoylecgonine, on the freshwater bivalve Dreissena polymorpha

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The increase in global consumption of illicit drugs has caused both social and medical problems, but also the onset of a potential new environmental hazard. It has been established that after human consumption, drugs and/or their metabolites end up in surface waters, after being carried through the sewage system, posing a potential risk for aquatic biocoenosis. However, even if many studies have showed the presence of several drugs and metabolites in freshwater in the high ng/L to low µg/L range worldwide, at present any information on their potentially harmful effects on non-target organisms is available. The aim of the present study was to investigate the cyto-genotoxic effects induced by the main metabolite of cocaine, the benzoylecgonine (BE), on a classical biological model as the freshwater bivalve Dreissena polymorpha. Our goal was reached through the application of a biomarker battery on zebra mussel hemocytes. The raise of genotoxic effects was investigated by the Single Cell Gel Electrophoresis (SCGE) assay, which evaluated primary DNA lesions, and by the DNA diffusion assay and the micronucleus test (MN test), which investigated fixed genetic damage. The Neutral Red Retention Assay (NRRA), by evaluating the lysosome membrane stability, was used to assess benzoylecgonine cytotoxicity. In addition, the activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and the phase II detoxifying enzyme glutathione S-transferase (GST) was measured in the cytosolic fraction extracted from a pool of entire bivalves in order to reveal a possible oxidative status unbalance of treated-specimens. 14 days exposure to two increasing nominal concentrations of benzoylecgonine (0.5 µg/L and 1 µg/L), comparable to those currently measured in surface and sewage water respectively, were performed under semi-static conditions. Our results highlighted that benzoylecgonine exposure induced significant ($p<0.05$) increases of both primary and fixed DNA damage at both the administered concentrations. In addition, since BE significantly ($p<0.05$) decreased the stability of lysosome membranes, our data also highlighted its cytotoxicity and the possible implications of oxidative stress for the observed genotoxic effects. Lastly, BE seemed able to induce moderate effects on the activity of antioxidant and detoxifying enzymes, as shown by the notable oxidative status unbalance of treated bivalves.

EP08A-5

Chronic effects of diclofenac on fish and mussels measured using human diagnostic techniques

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The non-steroidal anti-inflammatory drug diclofenac is thought to be one of the most toxic pharmaceuticals found in the environment and has been highlighted by the European Environment Agency as being of particular environmental concern. In the current study we have adapted human diagnostic testing techniques for use with environmental samples. Mussels (Mytilus spp.) and rainbow trout (Oncorhynchus mykiss) were exposed to 1 µg/L and 1000 µg/L diclofenac under semi-static conditions, with homogenised digestive gland supernatant (15,000g) sampled from the mussels after 7 and 14 day exposure and fish blood plasma sampled after 96h. Rainbow trout exposed to the environmentally relevant concentration of 1 µg/L diclofenac for 96h showed a significant increase in alanine aminotransferase (ALT) levels. ALT is commonly clinically measured as a part of a diagnostic evaluation of hepatocellular injury to determine liver health. Exposure to 1000 µg/L significantly decreased Alkaline phosphatase (ALP) levels, used in humans to indicated liver, bone and intestinal diseases. In the mussels diclofenac exposure showed a significant decrease in digestive gland ALT expression at both 1 and 1000 µg/L after 7 days. Aspartate aminotransferase (AST) also showed a significant decrease in expression after a 7 day 1000 µg/L exposure. All molluscs assayed to date have AST and ALT and both were previously found to be significantly inhibited by metal exposure in the clam Ruditapes philippinarum, where it was suggested as a useful biomarker for sublethal stress. Other endpoints measured include glucose, blood urea nitrogen, total bilirubin, and gamma glutamyltransferase. Many of these endpoints have been previously observed in fish and molluscs, but we are proposing their use to evaluate the effects of pharmaceuticals in the enviornment in a fast, efficient and standardised way using human diagnostic techniques and machines.

EP08A-6

Effects of environmental relevant concentration of pharmaceuticals on the immune system of Lymnaea stagnalis

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Municipal effluent is the most important contributor of pharmaceuticals to the environment; these pharmaceuticals do not occur as single contaminants, but as complex mixtures. The immune system could represent a target system of exposure to this class of toxicants, and Lymnaea stagnalis is likely to be exposed to these compounds. Thus, the major prevailing therapeutic classes (neurological, anti-hypertensive, antibiotic, hypolipemic drugs) were selected, representing compounds of critical concern for the aquatic environment. Their toxicity was tested as a mixture of environmental relevant concentration of each therapeutic class, as a global mixture of all selected pharmaceuticals and compared to the global toxicity of wastewaters coming from the effluent discharge of Montreal.

L. stagnalis were exposed in semistatic conditions to each mixture in triplicates (3 snails per replicate) during 3 days, and to surface water from the St Laurence River at upstream and downstream of the effluent discharge point. Hemolymph was collected and immunological parameters measured. Hemocyte count and viability was monitored, as well as phagocytosis activity, ROS and thiol production. Gene expression of genes involved the immune response (AIF, TLR4, MDM, SOD, Catalase, SeGPx, GSR, NOS and NOS bis) was also measured in real-time quantitative PCR.

Both the environmental concentration of pharmaceuticals mixtures, and the effluent-tainted surface waters modulated the immune response at expression and effect levels. All mixtures decreased hemocyte viability and count. Phagocytosis and ROS production was decreased except with the antibiotic mixture which increased them. All mixture increased thiols production, suggesting an increase of phase II biotransformation by pharmaceuticals. Except for TLR4, gene expression was generally decreased by the mixtures. Environmental relevant concentration of pharmaceutical mixtures modulate the immune response of L. stagnalis, the neurological mixture being the most potent, and the antibiotic one having opposite effects compared to the global mixture, but similar effects to the effluent.

The effluent decrased cell viability, but increased cell count. Phagocytosis, ROS and thiols production were increased. Gene expression was slightly increased and particularly TLR4. The effects of the effluent on the immune system of L. stagnalis can partly be only explained by the presence of some pharmaceuticals, but also by other factors as the bacterial load.

EP08B-1

Does the environmental risk assessment within the marketing authorization procedure ensure the environmental safety of Human Pharmaceuticals?

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The authorization of medicinal products consists of two phases: a pre-market and a post-market surveillance. For all new marketing applications of human medicinal products the European legislation requires an assessment of potential risks to the environment in the pre-approval phase. The respective European guideline on environmental risk assessment of human medicinal products came into effect in 2006 (EMEA/CHMP/SWP/4447/00) and an amending question and answers document in 2010.
The environmental risk assessment according to the EMA-guideline is a tiered process in which Phase I is an action limit approach only considering environmental exposure. If the predicted environmental concentration in surface waters (PECsw) exceeds the action limit of 0.01 µg/L, an in depth ERA based on studies on environmental fate and effects has to be performed in a Phase II. For estimating a potential environmental risk, the predicted environmental concentration (PEC) is compared to the predicted no effect concentration (PNEC). A PEC/PNEC ratio ≥ 1 indicate an environmental risk.
Since 2006 the Federal Environment Agency (UBA) assessed more than 700 marketing authorization applications. The major therapeutic groups assessed so far are antiinfectives, analgesics, psychotropics, cytostatics and hormones. Based on predicted and measured environmental concentrations a potential risk can be identified e.g. for some hormonal and psychotropic substances.
The presentation will illustrate the assumptions and uncertainties of the environmental risk assessment according to EMA guideline. Furthermore the legal provisions and the environmental needs are compared and discussed. To ensure a high standard for the environmental safety of medicinal products for human use a monograph system on active drug substances should be established. Only a pre-marketing monograph system on fate and effects data of drug substances in conjuction with an effective monitoring e.g. of the occurance of active substances in the environment within the post-market surveillance will be able to ensure the environmental safety of human and veterinary medicinal products in use.

EP08B-2

Prioritizing cytotoxic drugs present in aquatic systems and their occurrence in the environment

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Cytotoxic drugs are routinely used in chemotherapy to fight cancer; they work by disrupting critical cellular processes, often through a toxic mode of action. The cytostatics are discharged with wastewaters, largely through the excretion of the unmetabolized drug. This study considers the occurrence and behaviour of these chemicals in aquatic systems via prioritization of old and new anti-cancer agents based on their consumption, excretion and environmental fate. Commonly used cytotoxic drugs were listed based on accurate consumption data from a detailed hospital survey of the NW England, urinary excretion rates from clinical studies, their likely fate and persistence in aquatic, media as well as their reported presence in environmental wastewater. In addition to this, we report concentrations from WWTP influent, effluent and receiving waters across England. Samples were filtered and extracted using a combination of Strata-X and Florisil SPE cartridges in a LC-MS/MS analytical method. Cyclophosphamide, a commonly used cytotoxic drug, was detected at a maximum concentration of 22.7ng/L in wastewater effluent, with the limit of detection ranging between 0.03-0.12ng/L. This shortlist of cytotoxic drugs can be used for water screening programmes and ongoing work will involve the development of a multi-compound analytical method for the shortlisted chemicals based on a current published method for cyclophosphamide. The analytical method will then be applied in the development of a novel passive water sampler for these chemicals, which generally exhibit high aqueous solubilities and low KOW values. To date, only limited field studies have reported cytotoxic drugs in the aquatic environment and more extensive surveys are required to assess the distribution of these chemicals and the risk posed to aquatic biota and humans, especially in those areas where water is abstracted for potable water supply.

EP08B-3

Ecotoxicity from antimicrobials and analgesics during an influenza pandemic

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The global public health community has closely monitored the unfolding of the 2009-10 influenza pandemic to best mitigate its impact on society. However, little attention has been given to the impact that our response to a pandemic might have on the environment. Antiviral, antimicrobial and analgesic drugs prescribed to treat influenza and influenza-associated complications can be excreted into wastewater in a biologically-active form. Here we use a global spatially structured epidemic model to simulate the quantities of drugs used during an influenza pandemic. We couple this model to a wastewater and river flow model, to project drug concentrations in wastewater treatment plants (WWTPs) and receiving rivers in the Thames basin in Southern England. Ecotoxicologic modeling of species sensitivity distributions (SSD) for antibiotics (based on distributions of minimum inhibitory concentrations of antibiotics in pathogens), coupled to an assessment of their mixture toxicity, shows that projected concentrations of antibiotics in WWTPs and receiving rivers would not exceed toxicity thresholds in the case of a mild pandemic, as observed with the current H1N1 pandemic. However, at the peak of a moderate or severe pandemic, the mean antibiotic usage could increase by 13% and 252% as compared to inter-pandemic periods, respectively. Nearly one-third of the microbial community in 70% of the WWTPs in the Thames basin are projected to be growth-inhibited during a severe pandemic. A more severe pandemic might thus result in reduced WWTP efficacy, resulting in the release of partly untreated sewage into receiving rivers, leading to eutrophication, fish kill, and contamination of drinking water abstraction points. SSD modeling of the toxicity of analgesics to aquatic organisms in the Thames catchment shows that pandemic use is not likely to cause acute toxicity, however, a moderate and severe pandemic might lead to analgesic concentrations exceeding chronic toxicity thresholds for ibuprofen.

EP08B-4

Emission of human antibiotics and antineoplastics into the environment: identification of high risk exposure scenarios in Europe

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A screening tool has been developed for the identification of high risk exposure scenarios for humans and ecosystems due to the emission of human antibiotics and antineoplastics in Europe. The tool is based on country-specific consumption data, and its use results in a relative ranking of exposure scenarios. We used a spatially explicit approach. A data search was conducted on country- and substance-specific characteristics. These were combined with spatial data on the distribution of agglomerations and sewage treatment plants in Europe, and nation specific emissions were estimated. Calculations of the environmental fate of the antibiotics and antineoplastics were performed with the use of European spatial characteristics on a 100*100km grid scale, a spreadsheet-based multimedia fate model, and bioconcentration/biotransfer factors. In these fate calculations, special attention was given to the ionizing properties of the substances, especially those which are zwitterionic, i.e. fluoroquinolones and tetracyclines. Behavioral characteristics of four age-based target groups, i.e. infants, children, adults and elderly, were linked with the concentrations in exposure media derived from the fate calculations. Furthermore, we included specific consumption and activity patterns that could potentially cause high risks, e.g. the consumption of locally grown crops. Acute toxicity data from literature and publicly available databases were used for the ranking of the exposure scenarios for both aquatic ecosystems and humans. The study resulted in a set of rankings of aquatic and human exposure scenarios to antibiotics and antineoplastics on a European scale, and the scenarios most likely to cause risks for the aquatic environment or human health were identified. The rankings were incorparated in a set of GIS-maps to visualize spatial variations and can be used to identify worst-case exposure scenarios and to pinpoint those situations that should be given priority in further, more indepth, risk assessment studies. When data were (partially) lacking, worst-case assumptions were made. Consequently, scenarios for which data are lacking are likely to be assigned a higher rank. The outcome therefore gives an indication of the urgency of the existing gaps of knowledge in the assessment of these exposure scenarios.

EP08B-5

Antibiotic-induced change of bacterial communities associated with the copepod Nitocra spinipes

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Environmental pressures, such as physical factors, diet and contaminants may affect interactions between microbial symbionts and their multicellular hosts. Despite obvious relevance, effects of antimicrobial contaminants on host-symbiont relations in non-target aquatic organisms are largely unknown. We show that exposure to antibiotics had negative effects on survival and juvenile development of the copepod Nitocra spinipes and caused significant alterations in copepod-associated bacterial communities. Moreover, there were significant positive correlations between indices of copepod development and bacterial diversity, indicating that disruption of the microflora was likely to be a major factor behind retarded juvenile development in the experimental animals. As evidenced by ribotype distribution in the bacterial clone libraries, the exposure to antibiotics caused a shift in dominance from Betaproteobacteria to Cardinium bacteria; the latter have been shown to cause reproductive manipulations in various terrestrial arthropods. Thus, in addition to providing evidence that the antibiotic-induced perturbation of the microbial community associates with reductions in growth-related traits of the host, this study is the first record of a copepod serving as a host for endosymbiotic Cardinium. Taken together, our results suggest that (1) antimicrobial substances and possibly other stressors can affect symbiont-mediated interactions in copepods and other hosts, and (2) similarly to other arthropod species, reproductive biology of copepods may be affected by Cardinium bacteria.

EP08B-6

Ciprofloxacin toxicity in five marine species

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Recent publications and research indicate that input of pharmaceuticals to the environment is set to increase. Pharmaceutical products are particularly likely to cause effects on biological systems because that is what they are designed for. Most research so far has concentrated on freshwater interactions with very little marine data for use in risk assessments. Ciprofloxacin, a fluoroquinolone antibiotic, is found in many wastewater treatment effluents and could therefore conceivably make its way into the coastal marine environment.

This research has investigated the acute toxicity of Ciprofloxacin to five marine species: 7 day growth in the red algae Ceramium; 96 hour growth in the brown algae, Fucus; 72 hour growth in the green algae Isochrysis, 72 hour growth in the diatom, Skeletonema and 24 hour embryo development in the bivalve mollusc, Crassostrea. Tests were carried out according to standard OECD or ISO protocols where available, or from peer reviewed research papers. Results showed that for the most part Ciprofloxacin was not likely to be a source of concern in the marine environment with little or no toxicity recorded for most of the species. Most sensitive was Ceramium, with an EC50 of 10mg/l, with Skeletonema second. The oyster embryo assay showed little sensitivity.

The sensitivity of Ceramium and Skeletonema may be due to mode of action, or availability in the water column. A log P of 2.3 suggests weak but not exclusively lipophilic behaviour. Physiological differences between the algae, and the mollusc, may explain the difference in toxicity. Using this sort of actual marine data will make the creation of Marine Risk Assessments a more meaningful task.

EP08C-1

Geochronology of pharmaceuticals in coastal marine sediments

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Aquatic systems are subjected to the influence of wastewater discharges, which may contain significant amounts of organic contaminants. In spite of their low concentration in the water column, these chemicals may cause damage to the ecosystem in the long term due to chronic exposure. Among them, pharmaceutically active compounds (PhACs) have been the focus of many researchers within the last years. Most of these studies deal with their identification and distribution in water bodies. The present study shows the distribution and fate of PhACs in the sedimentary column, which offers a new insight that allows reconstructing the history of the contaminantion of an aquatic system by these compounds. A sediment core was sampled in 2008 in the deepest area of Jamaica Bay, a sewage impacted marine system heavily impacted by NYC wastewater treatment plants (WWTPs). The sediment core was sectioned at 2 cm intervals and dated by measuring 137Cs and 7Be. 70 different pharmaceuticals were analyzed in these samples, as well as linear alkylbenzene sulfonate (LAS), a widely used anionic surfactant. Anoxic conditions prevailing in the sediments favor preservation of organic contaminants. The profile of LAS concentration remains fairly stable following 1978, so any changes in the concentration of PhACs are interpreted as resulting primarily from changing inputs to the WWTPs surrounding Jamaica Bay. Only 16 of the 70 PhACs that were analyzed could be detected and their concentrations were much lower (< 50 ng/g) than those for LAS (up to 250 µg/g) in the same samples, mainly as a consequence of their lower production and consumption. Their sediment records are consistent with first use dates for pharmaceuticals. Thus, ibuprofen was first sold in 1974, and it has been very popular since then, so its presence could be observed in sediments from that date until today, showing an average concentration of 10 ng/g. Concentration of propanolol increased to 3 ng/g from 1977 to 1985, and then it decreased towards the surface of the core as it was substituted by metoprolol, which has improved its sales recently so its concentration rises from 5 to 35 ng/g over the last decade. Psychiatric drugs, such as carbamazepine or fluoxetine, could be also detected in spite of their relatively high solubility. Overall, the approach shown here allows monitoring the exposure of aquatic systems to PhACs during several decades and any changes that may occur in their use by nearby populations.

EP08C-2

Sorption and accumulation of pharmaceuticals in wastewater-irrigated fields in the Mezquital Valley, Mexico

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Due to population growth and urbanization, arable fields are increasingly irrigated with untreated wastewater worldwide. Environmental and health risks, such as proliferation and formation of antibiotic resistances, caused by pharmaceutical residues are profoundly affected by the compounds' binding and accumulation in soils. The Mezquital Valley north of Mexico City is the largest wastewater irrigation area facing these risks worldwide. We investigated the accumulation and sorption of the antibiotics ciprofloxacin, sulfamethoxazole, trimethoprim, clarithromycin, and the anticonvulsant carbamazepine in soils with different duration of wastewater irrigation (0-100 years). In contrast to our expectation, no accumulation of extractable ciprofloxacin and clarithromycin was detected. However, sulfamethoxazole and carbamazepine concentrations in the soils increased until a steady state of about 40 µg kg-1 soil (sulfamethoxazole) and 55 µg kg-1 (carbamacepine) was reached after approximately 15 years of irrigation. We hypothesized that this increase in concentrations with irrigation time was at least partly caused by a more efficient sorption of these compounds as a consequence of the accumulation of organic matter in irrigated soils. Sorption of sulfamethoxazole to soils irrigated for different periods of time was, however, weaker than sorption to non-irrigated soil. (De)sorption experiments indicate that a saturation of the soils sorption sites explains reduced sorption in irrigated soils compared to non-irrigated soil. Sorption of ciprofloxacin was always strong irrespective the soil organic matter content and the duration of wastewater irrigation. Strong sorption counteracts an accumulation of extractable ciprofloxacin in soil. Intermediate sorption leads to an accumulation of extractable sulfamethoxazole and carbamecepine in soil until an equilibrium between input with wastewater and dissipation is reached. Historical records of inputs allow an estimation of dissipation rates under field conditions. The saturation of sorption sites for sulfamethoxazol and carbamacepine during wastewater irrigation and interactions of these substances with other compounds contained in the wastewater increase their leaching to groundwater.

EP08C-3

Sorption and leaching behaviour of four emerging pollutants pharmaceuticals in agricultural soil

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Mobility of pharmaceuticals in soils is little known. These pollutants can be introduced in the soil by the wastewater irrigation practice that represents increasing interest nowdays. Traditional approach to study the mobility of organic pollutants taking into account the Kow parameter would not be appropriated for many pharmaceuticals because they are polar or ionic compounds, i.e. charged at wastewater and environmental pH for who an ionic interaction mechanism could be responsible for their retention by soils. Cationic exchange capacity (CEC) of the soil and other soil factors such as pH, organic matter content (OM), or presence of surfactants modified by the wastewater irrigation could influence the mobility of pharmaceuticals in soil. The aim of this study was to investigate the mobility of some frequently detected wastewater derived pharmaceuticals in soils under laboratory conditions. Four pharmaceuticals were chosen: carbamazepine(CBZ), venlafaxine (VEN) and their respective major human metabolites, 10,11-dihydro-trans-10,11-dihydroxyl carbamazepine (DIOL) and O-desmethyl venlafaxine (ODV). They are frequently detected in wastewater at concentrations higher than 1µg/L and they have different Kow and pKa values. The main results were: 1) batch sorption/desorption study showed that VEN and ODV were largely sorbed by two soils with low OM content and their desorption was dependent on the soil cationic exchange capacity. CBZ and DIOL were little sorbed by both soils and their desorption was not due to cationic exchange. The soil with greater CEC showed more affinity for all compounds. 2) leaching study on soil columns confirmed batch experiment with CBZ and DIOL leaching much more rapidly than VEN and ODV. VEN and ODV were only leached with 100 mM Ca2+ solution. No influence was observed with cationic and anionic surfactant solutions. Retardation of leaching was more important for the soil with greater CEC. This work showed a clear influence of soil CEC in sorption/desorption mechanism of ionic pharmaceuticals VEN and ODV. The Kow value by itself would not be able to predict correcly mobility of ionic compounds in soil. Other parameters related with CEC should be taken into account.

EP08C-4

Uptake of Pharmaceuticals into the Terrestrial Environment

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Increasing amounts of pharmaceuticals are being detected in soils. This is primarily due to sewage sludge being applied to fields as well as the use of reclaimed wastewater for irrigation, both of which contain high levels of pharmaceuticals which are then transferred to the soil. This study investigated the uptake and depuration of pharmaceuticals into earthworms (*Eisenia fetida*) with the ultimate aim being to develop models to improve the assessment of the risks posed by pharmaceuticals in the terrestrial environment. The studies were based on the OECD Guideline 317 and involved a 21 day uptake phase followed by a 21 day depuration phase. Studies were performed using radiolabelled compounds to allow for a lower limit of detection thus enabling environmentally relevant concentrations to be used. *E. fetida* and soil sampled throughout the experiment were extracted and analysed by liquid scintillation counting. Selected samples were also analysed by radioHPLC to assess whether any metabolism had occurred. The results from the study were modelled using OpenModel (v 1.2 Nottingham University) and based on a one compartment toxicokinetic model.

For both fluoxetine and diclofenac there was uptake within the first 6 hours of the experiment. The modelled uptake rate constants (K_{in}) show that fluoxetine had a higher overall uptake rate than diclofenac at 0.7428 L/kg d^{-1} and 0.1484 L/kg d^{-1} respectfully. When *E. fetida* were transferred to clean soil both compounds were seen to be

immediately eliminated from the organism, however modelled depuration rates were considerably slower than uptake rates for both pharmacreuticals. By the end of the depuration phase there were still traces of both pharmaceuticals in the earthworms. The modelling results were then used to evaluate existing quantitative structure-activity relationships used in environmental risk assessment.
Bioconcentration factors were estimated from the uptake and depuration rates. The diclofenac BCF was 167 and the BCF for fluoxetine was 133. Both of these values are comparable to BCF values estimated from equations used in risk assessment, such as the Technical Guidance Document. This research shows earthworms, *E. fetida*, can accumulate pharmaceuticals if they are present in soils at evironmentally relevant concentrations. Work is ongoing to assess the uptake of additional pharmaceuticals into earthworms, as well as to investigate how uptake can be influenced by soil parameters such as change in pH.

EP08C-5
The anti-arrhythmic drug flecainide: environmental detection and conserved mode of action in fish
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Flecainide is sodium channel blocker used to treat cardiac arrhythmic disorders in humans. We hypothesized that flecainide could cause adverse environmental effects, based on its sales and usage statistics, logP (4.0) and a presumably conserved mode of action in fish. We have therefore quantified levels of flecainide in environmental samples, and studied effects of the drug in rainbow trout (O. mykiss) in vivo and in vitro, using endpoints reflecting its known mode of action in humans. Using a triple stage quadrupole MS/MS TSQ Quantum Ultra EMR system, we could detect flecainide in treated sewage effluents (123 ± 17 ng/L), in surface water upstream (2.7 ± 1.2 ng/L) and downstream (36.8 ± 14.4 ng/L) from sewage treatment plants and in muscle of wild fish (0.16 ± 0.03 µg/kg). Rainbow trout were cannulated via the buccal cavity, and once a stable baseline was attained, stepwise increments (10x at each step) of flecainide were injected. Flecainide injections, equivalent to a human dose adjusted for body weight (2mg/kg), resulted in an acute significant decrease in heart rate and a tendency for an increase in arterial blood pressure. Mean flecainide level in plasma from injected fish was 0.9µg/ml, i.e rather similar to human therapeutic plasma concentrations (0.4µg/ml). Preliminary results from in vitro studies on twitch force and rate of contraction in rainbow trout ventricular and gastric strip preparations suggest decreases of both endpoints at levels close to human therapeutic concentrations. Taken together, this indicates at least a partly conserved mode of action between human and fish as well as a roughly similar potency, and the possibility of effects in non-target tissues. Waterborn dose-response studies, controlled bioconcentration studies and analyses of plasma levels in wild fish will allow for further assessment of environmental risks with flecainide.

EP08C-6
Accumulated antidepressants in wild-caught fish - a relaxed wildlife?
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Numerous recent studies have revealed pharmaceutical residues in the influent and effluent of waste water treatment plants and all kinds of surface water. In these studies mainly water samples have been collected which were concentrated by solid phase extraction prior chemical analysis.
On the other hand to deduce mechanisms of toxic action or to evaluate the toxicity of mixtures, and to interpret field data on bioaccumulated toxicants the tissue residue approach has been taken into account most recently. Therefore, we have focused in our study on a multi-residue analysis of polar to lipophilic pharmaceuticals from different trophic levels of the aquatic food chain (fish prey, fish, and fish eating birds).
Determined pharmaceuticals were the frequently detected ibuprofen (an analgetic), diclofenac (an analgetic), mefenamic acid (an anti-inflammatory drug), sulfamethoxazole (a bacteriostatic antibiotic), atenolol (a beta blocker), diphenhydramine (an antihistamine), diltiazem (a calcium channel blocker), carbamazepine (an anticonvulsant), fluoxetine (an antidepressant) and its main metabolite norfluoxetine.
Since our preliminary results have revealed exclusively residues of norfluoxetine, fluoxetine, diphenhydramine and carbamazepine in fish and fish prey the list of analytes were extended to other antidepressants (sertraline and citalopram) but also to more lipophilic pharmaceuticals like gemfibrozil, fenofibrate and naproxen or lipophilic metabolites of diclofenac (2-[2-(chlorophenyl)amino]benzaldehyde) and ibuprofen (2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid), which have been in parts detected in surface water.
Trace analysis of compounds was performed in a multi-residue method based on HPLC coupled to mass spectrometry (LC-MS) whereby identification and quantification of pharmaceuticals in biota were arranged in MS/MS mode. MRM transitions were classified in a different elution time window to increase measurement sensitivity.
The identified concentrations of analytes were ordinary higher in liver than in muscle tissue.

ET01 - A systems biology approach to predictive ecotoxicology

ET01-1
Network inference tools in ecotoxicology: application to Daphnia Magna
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Environmental protection agencies worldwide are presented with a huge challenge as a result of human innovation. Large numbers of chemicals are being released into the environment through a number of sources including chemical spills, sewage, industrial waste or agricultural run offs. Many of the underlying mechanisms of toxicologically relevent chemicals are currently unknown or incompletely characterized. "Omics"technologies have provided a high-throughput unbiased approach to address this issue. Statistical modelling approaches, identifying groups of features predictive of toxicity outcome have shown to provide informative results, especially when pathway knowledge is incorporated. This approach, however, is limited to the gene to gene interactions represented by the functional annotation. In this context, several reverse engineering methodologies have been developed to provide means of inferring the underlying regulatory network withour prior knowledge [1-3]. The resulting interactions can then be subjected to further methodologies to identify highly interconnected sub-networks or functional modules which can provide knowledge towards identifying novel adverse outcome pathways [4]. To demonstrate the effectiveness of these techniques we applied these methodologies to an mRNA expression dataset derived from Daphnia Magna exposed to sub-lethal concentrations of a number of ecotoxicologically relevant chemicals. We apply a well validated reverse engineering methodology, ARACNE [2], and identify functional modules which may provide knowledge towards understanding the response of this species to chemical exposure.
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ET01-2
Integrative monitoring of wetlands WWTP effluent remediation in the WIPE project
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Waterharmonica is a Dutch concept of natural filtration in constructed wetlands, applied to wastewater treatment plant (WWTP) effluent. Particles and nutrients are removed by aquatic organisms and reed beds, and the oxygen balance is restored, leading to positive effects on biodiversity. The WIPE project (Waterharmonica Improving Purification Effectiveness) was an extensive study of wetlands artificially constructed to remediate effluent from three wastewater treatment plants in The Netherlands. The main objectives of this project were to study the fate and toxicity of emerging organic pollutants from the WWTP effluents in order to assess if the discharged water meets the criteria of the Water Framework Directive (WFD) for good quality surface water. An extensive integrated monitoring program was applied at four positions each of three constructed Dutch wetlands, from raw effluent to discharged water. This program consisted of passive sampling, chemical analyses, bioassays and stickleback (Gasterosteus aculeatus) gene expression responses and fish ecology.
Several sampling campaigns were carried out to assess the chemical quality with a wide range of organic chemical analyses and a suite of in vivo and in vitro bioassays. Simultaneously, the ecology of the stickleback (survival, growth and reproduction) was studied by exposing the fish in mesocosms to water from four positions of the wetlands. Large variations in WWTP effluent quality were observed over time. At one of the wetlands an increased fish mortality was observed after exposure to the effluent, while a clear growth inhibition was observed in fish exposed in another wetland. No clear effects on reproduction success were observed in the surviving fish. Chemical analyses revealed that bioavailable levels of most organic micropollutants were not reduced, but a decrease in chemical toxic equivalents was observed after passage of the different wetlands compartments. Certain toxic responses (e.g. estrogenic endocrine disruption) also seemed to decrease in the wetlands. The in vitro ER-Luc measurements of XAD extracts seemed to correlate well with gene expression responses in stickleback. Less clear relationships between in vivo biomarkers and in vitro bioassays were observed for other parameters, such as cytochrome P450 1A (CYP1A) induction, thyroid induction and multi xenobiotic resistance (MXR) inhibition. The WIPE dataset is used to design a smarter WFD monitoring strategy for chemical water quality.

ET01-3

Transcriptomic responses of three-spined stickleback (Gasterosteus aculeatus) to wetland effluent remediation in the WIPE project

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The WIPE project was an extensive study of wetlands artificially constructed to remediate effluent from three wastewater treatment plants in The Netherlands. As part of this project, that also included microbiology, analytical chemistry and in vivo and in vitro bioassays, sticklebacks were exposed to water from the wetland systems in flow-through mesocosms, or maintained in a control mesocosm. Fish were hatched in the mesocosms and sampled after 1 year, with livers preserved for transcriptomics.
Based upon the stickleback genome sequence and results of optimization experiments, a 15,000 probe oligonucleotide microarray was designed for stickleback liver. Hepatic gene expression profiles were determined for 106 female and 75 male sticklebacks from 12 mesocosms. In males 3493 and in females 4290 transcripts were significantly differentially expressed (FDR<0.05), when comparing control and all exposed fish. The majority of genes differentially expressed between males and females were located on chromosome XIX, previously identifed as the stickleback sex chromosome.
When assessing a large dataset reflecting responses to complex mixed stimuli, such as WWTP effluents, individual biomarkers can be examined but more systematic approaches to data interpretation using prior knowledge can be valuable. As an example of biomarker response, at one sampling site expression of the three vitellogenin genes and estrogen receptor alpha were elevated in males. Extracts from passive samplers at this position elicited the greatest response in the ER-luc bioassay. Differentially expressed genes were characterised with respect to prior knowledge of their functions and their chemical regulators and disease associations (Comparative Toxicogenomics Database), resulting in hypothetical predicted profiles of chemicals and biological responses at each site. A number of changes correlated with liver weight, including repression of mitotic genes and associations with cholesterols and fatty acids. Responses were detected that are consistent with exposure to pollutants, such as PAHs, that reflected chemical data showing that some PAHs, including pyrene, exceeded PNEC values. Additionally, responses consistent with PCBs, oxidative stressors, endocrine disrupters and pharmaceuticals and personal care products were highlighted. Benefits and limitations of these approaches will be discussed.

ET01-4

Effects of metals and pesticides in Enchytraeus albidus - transcription, cellular energy allocation, oxidative stress biomarkers and reproduction: a systems biology approach

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An important challenge in terrestrial ecotoxicology is to unravel the modes of action of toxicants which are known to have a negative impact on ecosystems. *Enchytraeus albidus* is an important standard species in terrestrial risk assessment. This species is a typical inhabitant of a wide variety of soil types and fulfils important functions. Effects of chemicals in *E. albidus* have been addressed mainly assessing effects on their survival and reproduction. The present work comprises results of experiments using *E. albidus* where effects of the metals cadmium and zinc, and the pesticides dimethoate, atrazine and carbendazim were tested. Different concentrations and exposure durations were used as well as various endpoints from different levels of biological organization - gene expression, cellular energy allocation, oxidative stress markers, survival, reproduction and avoidance behaviour. The main goal was a systems toxicology approach where effects should be integrated. In concentrations known to cause a reduction on reproduction, all chemicals induced significant changes in the antioxidant enzyme activities and substrate levels and, in some of the conditions, oxidative damage was observed. In general, cellular energy allocation (CEA) results indicated reduced amount of energy reserves and disturbing of the mitochondrial electron transport system (ETS) by increasing the cellular respiration. This increase in the ETS activity corroborated the transcriptional evidences, where there was an induction of genes coding for proteins involved in the mitochondrial electron transport system. Gene expression analysis further enabled the identification of common mechanisms of toxicity and also key biological processes affected by each compound. The information gathered with gene expression analysis, along with the reduction of CEA and the verified oxidative damage on the membrane cells, can help to explain the decrease on the reproductive output at a later stage. It further indicated evidences of conserved mechanisms across-species. This presentation includes a combination of several studies that build along years and demonstrates the usefulness of integrating endpoints to better understand and predict the mechanisms of action of chemicals in a broader context.

ET01-5

High level dynamical models in Mytilus edulis

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Introduction. High-throughput omics including transcriptomics, proteomics and metabolomics could help in understanding an organism's response to various stresses. The acquisition and processing of such data for non-model species from their natural habitat is still a challenge, but would enable better comprehension of their response to pollution or seasonal changes.
Mussels, filter feeding sessile organisms living in the sea have been used for environmental monitoring and sessility makes them particularly suitable for studying the effects of chemicals in coastal regions.
Omics technologies alone provide large amounts of information that might prove difficult to interpret. Gene Ontology enrichment analysis can identify pathways that could be involved. Building static similarity networks helps further in finding groups of co-regulated genes possibly important in particular response. The strength of dynamic models relies in their ability to represent temporal changes, whether during natural cycles or in response to chemicals.
Approach. Here we present a systems biology approach of developing high level dynamical models of the annual cycle in blue mussel Mytilus edulis using 1H-NMR metabolomics data. Metabolites with similar temporal patterns were clustered and ordinary differential equation (ODE) based dynamical models were built between cluster profiles, using NIMOO [1]. This reveals potential time-dependant relationships between the clusters of metabolites.
Results. A dynamical model representing Mytilus edulis metabolite seasonal variation revealed differences between males and females. Perturbations of metabolite levels in Southampton identified clusters of metabolites that show feminising temporal patterns, suggesting the presence of feminising chemicals.
Importance. This work applies dynamic modelling approach to high-throughput data for understanding temporal changes in non-model species living in the wild environment.
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ET02 - Advanced statistical methods in quantative ecotoxicology

ET02-1

Statistical ecotoxicology - bright lines and dark alleyways

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Statistical modelling and inference plays a crucial role in ecotoxciology. Indeed, it could be argued that without the guiding principles of proper statistical design and analysis, ecotoxicology would be so severely handicapped as to be rendered ineffective. Of course there is a lot more to ecotoxicology than concentration-reposnse (C-R) experiments and the identification of 'safe' concentrations of contaminants in an ecosystem using statistical concepts such as the Species Sensitivity Distribution (SSD). However, given the prominence and importance of statistics as a critical element of the scientific process in ecotoxicological practice it is both timely and necessary to ask "how well are we doing?".
This paper attempts to anwser this question by providing an overview of the 'State of Statistics' (SoS) in ecotoxicology. In it, we briefly trace the history of involvement of statistics and statisticians and examine the development and population of the ecotoxicologists' statistical 'toolkit'. We reflect on what has worked well, what has let us down, and opportunities for future development.

ET02-2

Well past time to stop using NOELs and LOELs

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In the popular song, Hotel California, by the band the Eagles, "they just can't kill the beast". Th¬at is exactly our situation - we (and many others) have not yet been able to kill the "beast"that comprises No Observed Effect Levels (NOELs) and Lowest Observed Effect Levels (LOELs). Given that the concentration-response curve is one of

the founding paradigms of environmental toxicology it is absurd that hypothesis testing continues to be used to report toxicity. We submit that NOEL/LOELs do not meet the criteria of reporting concentration/dose response relationships. The only scientifically defensible measure of toxicity is the description of the concentration/dose response "curve". If toxicity tests are going to be research tools, then the data analyses must accurately describe toxicity. Since the 1970s the "curve"has been demonstrated to provide detailed information including the slope, the ECx values and the error term. Software is available that makes the computation straightforward. Bayesian curve fitting has been developed that allows estimation of a No Effect Concentration. The Bayesian curve fitting also provides credibility limits for the range of the curve. Since it is clear that the use of the "curve"is the only rational approach, we propose 5 recommendations: 1. Papers that rely on NOEL/LOELs should not be considered for publication in peer-reviewed scientific journals. 2. Curve fitting is the preferred model for concentration/ dose response relationships, ideally including confidence intervals and with supporting data. 3. NOEL/LOELs and similar data derivations should not be used as descriptions of concentration/dose response. 4. Papers that rely on historic NOEL/LOELs should only do so after every attempt has been made to replace these data derivations with concentration/dose response data. 5. Papers that treat NOEL/LOELs as data for further analysis such as the derivation of SSDs should receive extraordinary scrutiny. Regulatory agencies across the word must also begin a transition to the use of curve fitting to describe exposure-effect relationships. Although it is understood that for many chemicals NOELs/LOELs are the only results from some toxicity tests, the uncertainties in the accurate representation of the concentration-response curves result in unacceptable uncertainty, obviating informed decision-making.

ET02-3

Interpretation of toxicity data requires appropriate models for process and noise

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Quantitative interpretation of data requires models of some sort. In general, it requires a model for the process that has produced the observed data, as well as a model for the "noise"(the inevitable deviations between the process model and the data). Useful data interpretation requires a "mechanistic"process model, combined with a noise model that is appropriate for the observations. In (eco)toxicology, toxicity testing of compounds plays a central role. To analyse the data from toxicity tests, curve fitting is very popular. In many cases, this represents a situation with a poor model for both the process and the noise. The process model is just a description of the data that helps to interpolate to an ECx. For fitting the process model to experimental data, least-squares analysis is by far the most popular approach in this context. However, least squares represents a noise model, which comes with its own assumptions, which are almost always violated in practice. Toxicokinetic-toxicodynamic (TKTD) models are the most useful type of process model for analysing toxicity data. However, because these models fit the effect patterns over time, they require dedicated noise models. For quantal data such as survival, an appropriate model exists (the multinomial distribution). For continuous data such as body size and reproduction, more research is needed. In this contribution I want to raise the discussion about appropriate process and noise models in ecotoxicology, with an emphasis on the latter.

ET02-4

Estimation of critical effect concentrations: when choosing the right error model matters

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Ecotoxicology is in essence multidisciplinary and mathematical modelling is undoubtedly one of its main components. At a time when there is widespread recognition of the drawbacks of NOEC/LOEC values, statistical modelling approaches are particularly suited to estimating critical effect concentrations such as ECx or NEC values.
Generally, we can write:
$Y=f(X; \theta) + \varepsilon$
where Y represents the data; $f(X; \theta)$ is the deterministic part of the model (linear or non linear), with X the independent variables and θ the parameter vector; ε is the stochastic part, or the error model, chosen according to the data to describe randomness in realizations.
A review of modelling approaches used in ecotoxicology, or on routines dedicated to the estimation of critical effect concentrations (like the R-package drc), suggests that considerably more time and effort is devoted to the elucidation of the deterministic part of the model, and relatively little consideration given to defining an appropriate error structure. Nevertheless, the error model cannot be ignored for inference, model checking or prediction. Furthermore, based on our own publication experiences in the ecotoxicological literature has demonstrated that to the difficulty in convincing reviewers of the importance of the error model. The following correspondence from anonymous referees illustrates the point:
"*All the effort you put into deriving a sophisticated error structure of your model seems wasted*".
" *How important - in a practical sense - is it to get the error model right and the likelihood correct? How big is the difference if you would do the parameter estimation using a mathematically very correct approach (like you probably would do) or a slightly wrong, easy to use approach as I have done? How much would the parameters differ? I don't know how much, but I would like to know* ".
This is in fact the question we want to address in this paper: how influential is the choice of the error model in the quality of conclusions drawn about hazardous concentrations? With three examples extracted from the literature, we will illustrate the consequences of an inappropriate choice on point estimate of critical effect concentrations as well as on their confidence interval.

ET02-5

Variability in ecotoxicology: deliberate ignorance or just not getting it

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In the analysis of ecotoxicological datasets we tend to ignore the variability in the parameters being measured, fail to report known variances, fail to manage overall experiment-wise error rates and ignore the intrinsic value of variability in providing insight into biological responses to stressors. This is probably mostly a reflection of the relative youth of our field of science, a determination to find fundamental or simple answers to puzzling and complex issues and a desire to provide conservative numerical contaminant thresholds that satisfy the requirements of practitioners and regulators. Nonetheless, it does affect the practice of ecotoxicology in real-world situations and influences real world costs of environmental management.
In this paper we will examine the general lack of control of experiment-wise Type I error rates in multi-species and multi-sample assessments of toxicity, arguably the most common practical application of ecotoxicology in environmental assessment. While this may be a conservative trade-off that increases the statistical power to detect toxicity by an increase in the Type I error rate, actual measurement of statistical power or Type II error rates are rare in ecotoxicology, as is measurement of experiment-wise Type I error, so the value of this trade-off is rarely known. Modern SSD approaches compound this problem because endpoints from many studies are combined without consideration of the effect of compounded Type I errors on an overall model; a model from which, at least in the ANZECC/ARMCANZ framework, specific values of protective concentrations or hazardous concentrations are derived with, again, no overt reporting of error of the overall error rate. Furthermore, the test endpoints used as input to the SSD are commonly "standardised"by the use of application factors and acute to chronic ratios that are either arbitrary or derived from a limited number of tests without consideration of their variances.
The net result is that environmental management targets are specified that are based on layers of conservatism, and that have error rates that could be measured and reported, but generally are not. How much this costs society and operators in terms of compliance with overly-conservative guidelines is not known and incalculable. We believe that it is possible to better report and measure these hidden error rates, and that better insight into them may well provide for more cost-effective management.

ET02-6

Statistical evaluation of experimental designs for modelling of concentration-response functions in ecotoxicology

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Modelling concentration-response curves is now a classical way of analyzing ecotoxicological bioassays, which often replaces the severely criticized NOEC approach. However, most ecotoxicological bioassays are conducted according to designs appropriate for hypothesis testing but not for regression analysis implied in a modelling approach. The adaptation of experimental designs to regression analysis was rarely discussed in ecotoxicology. The use of D-optimal criterion to develop cost-effective designs for ecotoxicological bioassays was recently proposed. But the application of such local optimal designs requires a prior choice of the model, and a good prior knowledge of the values of the model parameters. This is a strong limitation on their applicability for defining standard protocols for ecotoxicological bioassays. In fact the prior knowledge of parameters is often approximate and a local optimal design based on wrong parameter values may be bad. Moreover, calculated optimal values for concentrations may be difficult to experimentally reach, or too precisely defined in regard to measurement precision of the concentration of the studied compound.
While evaluating the optimality of an experimental design, instead of using fixed values for model parameters, one could use a prior distribution for model parameters, summarizing the possible values the parameters may take, according to the prior knowledge of the ecotoxicologists. We performed simulations based on such prior distributions in order to evaluate standard and modified designs for ecotoxicology, taking the example of 21-day Daphnia magna survival bioassays. In such bioassays, tested

concentrations are generally defined following a geometric sequence, often of common ratio 2, centered on the expected LC50 value guessed from prior knowledge. The same number of individuals is generally tested for each concentration and for the control group. We quantified the impact of the total number of tested organisms and of the common ratio of the geometric sequence (for a fixed total number of organisms) on the precision of LC50 and LC5 estimations. Results may help ecotoxicologists to choose the more appropriate design and fix the total number of tested organisms, depending both on their prior knowledge about the LC50 value and on their expectation about precision of estimates.

ET03 - Animal alternatives: methods, endpoints and testing strategies

ET03A-1

An updated assessment of the relationship between the Zebrafish embryo toxicity test and the OECD 203 fish toxicity test - what can we say now?

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An integral aspect to the development of the Zebrafish Embryo Toxicity Test (ZFET) is the determination of its relationship to the OECD Acute Fish Toxicity Test Guideline (TG 203). The most recent update (Lammer et al. 2009) included comparisons of ZFET to the five most commonly tested species in the OECD 203. Since 2009, a large increase in the literature has occurred and a major validation study been conducted (20 chemicals performed in triplicate in 3-7 different laboratories). An updated assessment is timely. We review the data as of 2009, additional information available today, and provide new assessments. ZFET studies have increased to >500 on approximately 200 chemicals. OECD 203 data are available for approximately 150 of these compounds representing about 1250 individual toxicity tests. Fifteen major chemical categories are found in the database including industrial chemicals, agrochemicals, pharmaceuticals, feed stuffs, biocides, polymers, inorganics, and metals that spanned 9 orders of magnitude. Orthogonal regression was used to compare ZFET-OECD 203 overall and the resulting regression had a slope of 0.99, intercept of -0.03, and a correlation >0.9. We used class-specific QSARs to compare predictions for chemicals tested in ZFET and the OECD 203 (this provides perspective for chemicals that lack matching fish data). Fish QSARs provided equivalent predictions for fish embryos (e.g., for neutral organics, ZFET and fish predictions had equal levels of departure from the standard QSAR published in USEPA ECOSAR. Overall, the addition of new data to the ZFET-Fish database continued to improve the regression relationship, markedly expanded the domain of tested chemicals, and provided evidence that future QSAR developments will be useful.

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"Disclaimer: The opinions expressed and the arguments employed herein are those of the authors and do not necessarily reflect the official views of the OECD or of the governments of its member countries, or the European Commission'"

ET03A-2

Determination of internal chemical concentration in zebrafish (Danio rerio) embryos - a toxicokinetic approach

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The zebrafish (*Danio rerio*) embryo is an established animal replacement model organism in toxicology. A broad range of toxicological applications of zebrafish embryos is described in literature. In these applications the observed effects are usually linked to the ambient exposure concentration. But the internal concentration might reflect the biological effective dose and therefore provide access to describe biological potency of a chemical.

Several studies using radioactively labelled compounds for the determination of the internal chemical concentration in zebrafish embryos are published. For a broader application an approach without the use of radioactively labelled compounds could be useful. Therefore an approach for the determination of the internal chemical concentration in zebrafish embryos without the use of radioactively labelled compounds is introduced here.

The time dependent internal concentration of compounds with different $\log K_{ow}$ was measured at different exposure concentrations in zebrafish embryos. A one compartment first-order toxicokinetic model was used in order to estimate uptake and elimination rate constants. Data of fluoranthene as model substance are exemplarily shown here.

In this study, zebrafish embryos were exposed to an aqueous solution of one test substance in a static but agitated system under controlled conditions. Samples of exposed embryos were taken at 8 time points between 3 to 72 hours of exposure. Fluoranthene was extracted from whole embryos and analysed with HPLC-FLD/DAD. The concentration-time profiles were assessed.

Uptake rate constants (k_1) and elimination rate constants (k_2) were estimated by fitting the time course of internal concentrations with a one compartment first-order toxicokinetic model. There was no concentration dependence of the bioconcentration factor observed. This agrees with the assumption of a simple first-order uptake and depuration model.

In conclusion, the determination of the internal chemical concentration in zebrafish embryos without the use of radioactively labelled compounds was realized successfully and is recommended for a broader application. Using the one compartment first-order toxicokinetic model for this study, the suitability of the model performance and the experimental data is demonstrated.

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ET03A-3

Single-compound exposure versus co-exposure approaches in the in vivo EROD assay with the zebrafish (Danio rerio)

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This study is part of the joint research project "DanTox"funded by the German Federal Minister of Education and Research (BMBF) and aims at developing eukaryotic test systems to investigate ecotoxicological effects of contaminated sediments. The visualization of specific activation of the Ah-receptor directly in the zebrafish (Danio rerio) embryo via live imaging is one of these bioassays. The measurement of ethoxyresorufin-O-deethlyase (EROD) activity is regarded as a highly sensitive indicator of specific planar polycyclic aromatic hydrocarbons (PAHs) and structurally related compounds. CYP-induced fluorescence signals result from the ability of CYP1A to convert 7-ethoxyresorufin (7-ER) to auto-fluorescent resorufin. The transparency of the zebrafish embryo allows direct detection of EROD induction via epi-fluorescence microscopy. The co-occurrence of agonists and inhibitors of CYP1A is typical of complex environmental mixtures and required modifications of the in vivo EROD assay. Therefore, zebrafish embryos were used in order to evaluate the EROD-inducing potential of common single-compound exposures in parallel with binary mixtures of each compound (or sediment extract) and the PAH-type AhR agonist β-naphthoflavone. For chemical testing, the following substances were selected: methyl mercury(II)chloride, chlorpyrifos, Aroclor 1254, bisphenol A, 2,3-benzofuran, quinoline and β-naphtoflavone as positive control. The sediment samples were collected from three sites: two from the Rhine River (Altrip and Ehrenbreitstein) and one from the Vering Canal in Hamburg.

Due to their molecular structure, methyl mercury(II)chloride, chlorpyrifos and bisphenol A failed to induce EROD. More precisely, chlorpyrifos turned out to be a strong CYP1A inhibitor (< EC10 value) and caused typical edema-related toxicity. Aroclor 1254 as well as the heterocyclic compounds quinoline and 2,3-benzofuran showed a particularly short temporal induction pattern and lead to inhibition of the CYP1A catalytic activity in a concentration- and time-dependent manner. In order to avoid fluctuations in CYP1A activity due to prolonged exposure with chronic effects as a consequence, the exposure time was reduced to 3 h, which resulted in a stronger, less variable and more sensitive EROD response. Pretests with the embryo toxicity test suggest the presence of AhR agonists in the sediment extracts; further testing is on-going.

ET03A-4

Improving the capacity of zebrafish embryos for predicting acute fish toxicity - analysis of neurotoxic endpoints using the photomotor response (PMR)

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Fish embryo toxicity exhibits a high correlation with acute fish toxicity. However, some neurotoxic compounds appear to represent outliers in this correlation. These compounds either do not provoke mortality at all (e.g. flucythrinate and esfenvalerate) or at levels well above those for acute toxicity in adults (e.g. azinophosmethyl or propoxur). We hypothesized that the neurotoxic mode of action is not leading to lethality in embryos but that measurement of sublethal indicators of neurotoxicity would allow predicting the effects in adults. Therefore, the photomotor response (*PMR*, Kokel, Nat Chem Biol 6, 231-237) was analysed as a potential indicator of neurotoxicity. The *PMR* is a characteristic, quantifiable movement pattern of the embryos which is triggered through an intense light stimulus. The alteration of the movement pattern by exposure to chemicals was shown to correlate with the mechanism of action. The *PMR* measurement was conducted using video recording and image analysis with a commercial system. After an optimization process we were able to detect a concentration dependent change in the *PMR* - patterns. We determined the *PMR* of two AChE inhibitors (azinphos-methyl and propoxur), two voltage gated sodium channel agonists (fluycthrinate and esfenvalerate) and one voltage gated sodium channel antagoniste (tricaine). Thereby the polar narcotic substance 3,4-dichloranailine was used as a non-neurotoxic reference compound. By comparing a motion index (pixel changes within a 20 second analysis window), of exposed embryos (exposure from 2-33/37 hpf) to a control, increased or decreased movement was determined and visualised by a barcode

which was further used for cluster and PCA analysis.
Within a certain concentration range *PMR* pattern of neuroactive molecules clustered in accordance with their mode of action. At highest tested concentrations effects on the *PMR* was most likely caused by secondary effects rather than by direct neurotoxic effects (around LC_{10} of embryos). Application of an effect-index for the whole measurement or a specific response phase allowed a concentration response modelling and calculation of EC_{50} values. The EC_{50} of neurotoxic compounds were found in the same range of concentrations as effect levels for acute toxicity in adult fish. The data indicate that including neurotoxic endpoints may improve capacity of the zebrafish embryo test for predicting acute fish toxicity.

ET03A-5
Looking for estrogen mimics using transgenic tg(cyp19a1b-GFP) zebrafish embryos
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The tg(cyp19a1b-GFP) transgenic zebrafish expresses GFP under the control of the cyp19a1b gene, encoding brain aromatase. This gene has two major characteristics: (i) it is only expressed in radial glial progenitors in the brain of fish and (ii) it is exquisitely sensitive to estrogens. Based on these properties, we demonstrate that natural or synthetic hormones (alone or in binary mixture), including androgens or progestagens, and industrial chemicals induce a concentration-dependent GFP expression in radial glial progenitors. As GFP expression can be quantified by in vivo imaging, this model represents a very powerful tool to screen and characterize compounds potentially acting as estrogens mimics either directly or after metabolization by the zebrafish embryo. This study also shows that radial glial cells that act as stem cells are direct targets for a large panel of endocrine disruptors, calling to more attention regarding the impact of environmental estrogens and/or certain pharmaceuticals on brain development. Altogether these data identify this in vivo bioassay as an interesting alternative to detect estrogen mimics in hazard and risk assessment perspective.
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ET03A-6
Refining the 48h-zebrafish embryo test - a mode of action dependent approach
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In the process of developing alternatives to animal based toxicity tests, the fish embryo toxicity test (FET) has gained significant attention in recent years. Great efforts have been made to extend the scope of application for zebrafish embryos in particular. Consequently, many genomics and post-genomics methods have found their way into fish embryo studies to address ecotoxicological questions beyond acute toxicity. As for many substances, pesticide effects on the zebrafish embryos often occur only outside the time frame of the standard ZFET to date or on submorphological levels. The recently revised European directive on the protection of animals used for scientific purposes now specifically tolerates the use of not yet independently feeding embryonic and larval stages of non-human vertebrates for testing, what will further promote the development of methods to broaden the scope of the ZFET within the regulatory framework. Here we present the assessment of pesticide toxicity on the zebrafish embryo on different developmental stages and different levels of organization. For thirteen insecticides we conducted a standard FET (48h) and an extended FET (120h). A selection of the tested substances was subjected to whole-genome expression analysis. Correlation was better between D. rerio embryo EC50 values and adult O. mykiss acute toxicity values than for lethal FET effects only. Adult O. mykiss acute toxicity was more sensitive than embryo toxicity. For many chemicals tested, the first 48hpf were found not to be the most susceptible stage. Morphological effects at 48hpf often comprised minor, non-lethal effects. Exposure to the same concentrations but beyond 48h led to more severe and lethal effects. Chemicals used in microarray experiments induced differential gene expression at the EC20 or below. Pesticides of different MoAs displayed different numbers of differentially expressed genes. Pesticides with functionally similar MoAs shared a higher number of differentially expressed genes than those with dissimilar ones. Regulated genes at 48h related to molecular functions, which can be linked to the morphological effects and to the given MoA. The inclusion of every observable morphological effect and the extension of test duration can render a more realistic estimation of the adult toxicity than a standard 48h ZFET. Further, different transcriptome responses may reveal, additional test data provided, MoA specific transcriptional signatures or gene markers.

ET03B-1
A novel animal replacement system for the detection of endocrine disruptive capabilities in sexual development
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Alternatives to animal testing for prediction of local toxicity and genotoxicity have been recently established. However, currently these methods are not suitable for measuring endocrine effects in developing organs such as e.g. embryonic gonads. Here we present a phenotypic anchoring of a comprehensive study on sex-specific gene expression analysis accompanied by histological analysis of endocrine disruption in chicken embryo gonads, having the potential for an animal replacing system for endocrine disruptive toxicologic and ecotoxicologic examinations of chemicals.
Chicken embryos were inoculated with different amounts of tributyltin (TBT) and bisphenol-A (BPA). Embryos were incubated and their gonads analyzed histologically 2 d prior to hatching. From identically treated embryos right and left testes and ovaries were separated and genome-wide transcription profiles generated using SuperTag Digital Gene Expression (ST-DGE, SuperSAGE) profiling.
Male and female gonadal tissues both revealed histological aberrations in response to TBT and BPA. Female gonads became masculinized in response to TBT and, vice-versa, BPA-treated male gonads underwent feminization. Moreover, in female gonads inoculated with BPA clearly visible structural aberrations occurred. Following exposure to both chemicals mortality increased especially in the most affected sex (TBT: females, BPA: males). The expression profiles of more than 60 million mRNAs revealed massive effects of both chemicals, TBT and BPA, on important cellular signaling pathways. Gene expression differences were most pronounced in the phenotypically most affected sex.
Our results demonstrate that endocrine disruptive chemicals exert their effects on several levels including but not restricted to known hormone-based pathways. Together with an ongoing study of gene expression differences in very young life stages and different chemicals these data will form the basis for a blow-by-blow analysis of sex-specific gene expression during embryonic development. The ongoing project aims at the development of an in vitro method for testing chemicals in chicken eggs for replacement of juveniles and (sub-) adult rodents using stages with no pain perception. This should allow a highly sensitive assessment of modes of action of chemicals, which might show consequences in the next generations.

ET03B-2
Assessment of the impact of an abandoned dump site on the health of adjacent soil ecosystems by in vivo and in vitro testing with Eisenia fetida
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Earthworms are considered as sentinel organisms for soil health assessment, and among them, Eisenia fetida has been selected for the development and application of standard tests due to its sensitivity and easy maintenance. E. fetida has been successfully employed for real soil assessment through biomarker measurements. Recently, an in vitro test with E. fetida coelomocytes was developed, including in vitro exposure to metals and elutriates of standard and real soils contaminated with pollutants alone or in mixtures. In the present work, biomarkers were determined in vitro in coelomocytes after both in vivo exposure (soils) of earthworms and in vitro exposure (soil elutriates) of coelomocyte explants extruded from stock healthy earthworms. The in vitro responses after in vitro and in vivo exposures were compared, aimed to replace the in vivo exposure approach in the future and contribute thus to reduce the use of animals in soil toxicity testing. For comparisons, two biomarkers (NRR and riboflavin content) were used for ecosystem health screening in soils surrounding an abandoned dumpsite. The selected biomarkers indicated a decrease in coelomocyte viability and reduction of riboflavin content after exposure to the most polluted soils around the dumpsite (in vivo exposure) and their elutriates (in vitro exposure). The high toxicity of soils was confirmed after 14 d in vivo exposure, as 100% mortality was recorded. It was concluded that in vitro testing of coelomocytes after in vivo and in vitro exposure methods provided similar information about the health of soils surrounding the dumping site. The selected cellular biomarkers were able to discriminate between polluted and unpolluted soils, even at low pollution levels. Therefore, when biomarker endpoints such as NRR capacity and riboflavin content are measured in vitro, the in vitro exposure approach is suitable to replace the in vivo exposure assays for soil health assessment.

ET03B-3
Genotoxicity of uranium in embryonic zebrafish cells
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Uranium is a heavy metal used in the nuclear industry and for military applications. Studies on mammals have shown that uranium is genotoxic but up to now the major DNA repair pathways activated by uranium remain to be analyzed. Particularly, the repair of double strand breaks (DSBs) remains to be addressed in aquatic vertebrates such as zebrafish. In this study we analyzed molecular level effects of exposure to depleted uranium in embryonic zebrafish fibroblasts (ZF4 cells). We assessed DNA damage and the activity of the major DSB-repair pathways, i.e. non-homologous end-joining (NHEJ) repair, after a 24 hours exposure to uranium concentrations ranging from 1 to 250 µM, by immunodetection of the phosphorylated variant of histone H2A member X (γ-H2AX) and the DNA-dependent serine/threonine protein kinase (DNA-PK). DNA Single Strand Breaks (SSBs) were also detected using the alkaline comet assay. Cytotoxicity and bioaccumulation were measured for each exposure condition. Additionally, the localization and ultrastructural effects of internalized uranium within ZF4 cells was determined using transmission electronic microscope (TEM-EDX).
Exposure to uranium results in the production of DSBs a few hours after incubation. These breaks trigger the phosphorylation of H2AX proteins. We showed that the DNA-PK kinase activity, essential for NHEJ, is altered by the presence of uranium. The presence of uranium in cells disturbs but not inhibits the repair rate of DSBs. Such a result suggests an impact of uranium upon the reparability of DSBs and the potential activation of alternative DSBs repair pathway leading to the propagation of possible misrepaired DSBs. In parallel, we observed the formation of precipitates in lysosome-like vesicles for 250 µM of uranium in the medium . The appearance of these precipitates is concomitant with the decrease of the number of DSBs per cell. This process might be a part of a defence system whose role in counteracting cytotoxicity calls for further dedicated research.

ET03B-4
Mapping ethynylestradiol (EE2) effect on gene and protein expression in in vitro and in vivo exposed rainbow trout (Onchorhyncus mykiss)
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Evaluation of *in vitro* methods performance compared to *in vivo* are crucial for implementation of the 3R`s (reduction, refinement and replacement) in ecotoxicological testing. *In vitro* models such as continuous cell lines and primary cell cultures are well characterised high-throughput screening tools for several compounds including endocrine disruptors [1,3]. Use of different *in vitro* models has provided knowledge about the different modes of action (MoA) and the potency of chemicals that induce perturbations of natural endocrine action. Endocrine disruptors such alkylphenols, ethynylestradiol and bisphenol A act as estrogen mimics by binding to and activating estrogen receptor (ER)-mediated cellular responses. As *in vitro* methods lack some of the organ complexity and toxicokinetics provided by whole organism models, these methods may not adequately address the multitude of cellular responses occurring after chemical exposure. The objective of the present study was to determine whether primary hepatocytes from rainbow trout (*Oncorhynchus mykiss*) can represent a feasible alternative to use of *in vivo* testing of environmental ER agonists. *In vitro* and *in vivo* cellular responses were characterized after exposure to the model estrogen ethynylestradiol (EE_2) by a combination of global gene expression and assessment of single gene and protein expression. In obtained microarray results, fish exposed *in vivo* to EE_2 had a significant up- (1674) and down (1449) regulation of totally 3123 genes where vitellogenin was significantly up-regulated (3600 times). The vitellogenin gene and protein expression was also highly induced in the EE_2 exposed primary hepatocytes, showing a concentration/time dependent relationship. Results obtained from the *in vivo* and *in vitro* model shows similarities in response on the gene and protein level of Vtg when exposed to EE_2. In conclusion, the rainbow trout primary hepatocytes have shown to be a promising tool for characterising estrogenic response *in vitro*. Due to the probable preservation of innate cellular properties, these cells may represent a versatile screening for various compounds.

ET03B-5
Predicting chemical concentrations in fish and fish cells to account for internal exposure in toxicity assessment
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Environmental regulations require comprehensive testing and risk assessment before a chemical can be approved for use. In ecological risk assessment of chemicals in water, fish play a very important role, being the only vertebrate representative of freshwater systems. Quantification of chemical toxicity is generally based on measurements of external exposure; however, in order to understand, interpret and extrapolate toxicological effects, using internal concentrations of chemicals is more suitable. For this reason we need to understand the relationship between the external and internal concentration of chemicals in fish as well as in in vitro systems, and to quantify internal concentration of a chemical using toxicokinetic (TK) modelling. Therefore in this study we aim to: predict chemical concentrations in fish, measure and predict chemical concentrations in fish cells, link chemical concentrations in cells to the effect on cells and link the effect on cells to the effect on fish.
To predict chemical concentrations in fish we used a Physiologically Based Toxicokinetic (PBTK) model. Based on the PBTK model, internal concentrations of organic chemicals were predicted and compared with measured internal concentrations in rainbow trout. To measure chemical concentrations in cells, we exposed a rainbow trout gill cell line (RTgill-W1) to the radiolabelled chemicals for 1, 2, 4, 8, 16 and 24 h at 19 °C. Amounts of chemicals were then measured in medium, plastic well and cells.
Results demonstrated a clear relationship between predicted and measured internal concentrations in fish. Statistical analyses also confirmed a good agreement between measured and predicted values (coefficient of determination, factor_10 and the general distance between measured and predicted values were 0.79, 95% and 3.54, respectively).
The distribution of a chemical in a well plate differed for different compounds. For instance, the difference between Pentachlorophenol (PCP) and Malathion (Mal) can be caused by different log KOW of these compounds (PCP: 5.12, Mal: 2.36). In addition, we found that the concentration of a chemical in cells is time-dependent.
Thus, both approaches, PBTK modeling and experiments on fish cell lines, can be used to obtain internal concentrations of various chemicals which open applications in ecotoxicology and ecological risk assessment.

ET04 - Bioavailability and bioaccumulation - impact of environmental, biological and ecological variation

ET04A-1
Problems faced when evaluating the bioaccumulation potential of substances under REACH
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The identification and regulation of substances which combine persistency, bioaccumulation potential and toxicity (PBT-substances) is one central aspect of the European chemical legislation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals). PBT substances may elicit adverse long-term effects after release to the environment. The determination of a substance having PBT-properties is based on a set of distinct cut-off criteria identified in Annex XIII of the REACH regulation. Regarding the bioaccumulation potential the evaluation is focused on the substance´s bioconcentration factor (BCF) as a decisive criterion. The REACH guidelines provide a selection of standardised test procedures for measuring BCF and guidance in appraising test results. Additionally, alternative test results such as bioaccumulation factors (BAF) and biomagnifications (BMF) as well as additional indications for a bioaccumulation potential such as trophic magnification (TMF) derived from field studies may be considered for the assessment using a weight-of-evidence approach. However, the assessment of bioaccumulation will mainly depend on standardised tests. The currently used test systems with aquatic exposure have been demonstrated to generate reliable results for the majority of neutral, lipophilic organic substances, which facilitate a clear decision making by means of the crucial BCF cut-off criteria of Annex XIII. However, certain substance groups such as highly hydrophobic organic substances, amphiphilic and non-lipophilic compounds are difficult to evaluate following common test strategies due to inappropriate test systems or accumulation mechanisms not based on lipophilicity. Recent scientific progress has already been made to establish alternative test systems and to refine the bioaccumulation assessment by consideration of additive accumulation mechanisms and indications. This presentation aims to give an overview on actual shortcomings in the current bioaccumulation assessment under REACH.

ET04A-2
Nestling predatory bird feathers reveal ecosystem-dependent bioaccumulation of persistent organic pollutants
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Bioaccumulation levels and patterns of persistent organic pollutants (POPs) in predatory birds are often confounded by individual biological variation, such as sex, age, diet and migration. Therefore, as food ingestion is a major POP exposure pathway, exposure assessment studies increasingly incorporate carbon (C) and nitrogen (N) stable isotope analysis (SIA) to quantify confounding variation in feeding ecology.
In this study, we hypothesised that the study of nestlings minimises confounding of certain biological variables, such as age, metabolic capacity, migration and reproductive capacity. We additionally hypothesised that POPs behave differently in marine and terrestrial ecosystems. To test these hypotheses, we analysed body feathers from nestling Northern Goshawk (NG; Accipiter gentilis), Golden Eagle (GE; Aquila chrysaetos) and White-tailed Eagle (WTE; Haliaeetus albicilla) to relate POP bioaccumulation to feeding ecology, i.e. carbon source (δ13C) and trophic level (δ15N), and investigate how relationships may differ between species foraging in different ecosystems.
NG, GE and WTE nestling body feathers all contained quantifiable levels of Σ PCBs (mean: 55.95, 36.36 and 73.83 ng g-1), p,p'-DDE (mean: 48.56, 30.34 and 22.07 ng g-1) and Σ PBDEs (mean: 3.08, 1.00 and 5.78 ng g-1). Generally, the bioaccumulation profile suggests that marine ecosystems are a source for PCBs, terrestrial ones for p,p'-DDE, and that vicinity to urbanised regions influences levels of PBDEs.
δ13C and δ15N were positively correlated in nestling WTE feathers ($r = 0.589$; $P = 0.001$), but not in the terrestrial species ($P \geq 0.094$). This suggests differential C and N

cycling in terrestrial and marine ecosystems and is prominently reflected in correlations between SI and POPs. Firstly, while δ15N and POPs correlated significantly in all species, correlations between δ13C and POPs were only significant in the marine foraging WTE (-0.449 ≥ r ≥ -0.388). Secondly, in contrast to negatively correlated δ15N and POPs (-0.426 ≥ r ≥ -0.377) in WTE, correlations in NG and GE were positive (0.450 ≤ r ≤ 0.697).
Our results show that variable bioaccumulation patterns between marine and terrestrial foraging predatory birds are related to species-specific feeding ecology. Furthermore, although studying nestlings minimises confounding of certain biological factors, we will further investigate how sex and body mass variation may additionally explain bioaccumulation differences.

ET04A-3
Fate and bioavailability of insecticides across DOM-gradients in model ecosystems with biofilms and primary consumers
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1. Introduction
To better understand the impact of pesticide contamination on aquatic ecosystems we need fundamental information on how pesticides enter food webs and interact with organisms at different trophic levels.
2. Materials and methods
We used model ecosystems with cultivated biofilms and snails to quantify the fate and bioavailability of insecticides with different hydrophobicity (carbofuran, lindane, chlorpyrifos) across a DOM concentration gradient (low, medium, high). Pesticide pulses were added at the start of the 72-h experiments. Treatments without tiles and biofilms were run to quantify passive uptake. Snails were allowed to feed for 48 h. This set-up mimics the mechanisms at the base of food webs that act during runoff-events in agricultural streams.
3. Results and discussion
Partitioning to biofilms reduced chlorpyrifos water concentrations by up to 10±1.0% at high-DOM, but was noticeably higher at low-DOM than at medium-DOM. Conversely, lindane water concentrations were 17±0.7% higher in the presence of biofilms for all DOM-treatments.Pesticide BCF for biofilms were affected by both DOM and pesticide type, due to differences between lindane and chlorpyrifos treatments. BCFs for snails were only affected by the type of pesticide, and nearly 60% higher for both lindane and chlorpyrifos than for carbofuran.Bioaccumulation factors (BAF) between snails and biofilms showed differences across DOM-gradients for lindane and chlorpyrifosThe lack of concentration-dependent effects of DOM on pesticide fate and bioavailability suggests that also DOM-quality affects these end points. Although the share of pesticides recovered in biofilms was consistently less than 1%, their concentrations in biofilms were more than 75- (carbofuran) and 382-times (lindane) higher than those in water.
4. Conclusions
- The high and efficient sorption of lindane, and chlorpyrifos show that biofilms may act as a sink for pesticide peak concentrations during run-off events.
- DOM and biofilms in aquatic environments affect the fate of pesticides following runoff-events and their incorporation in aquatic food webs.

ET04A-4
Bioaccumulation and biomagnification of POPs in species from the Scheldt estuary (Belgium)
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As persistent organic pollutants (POPs) are spread worldwide over various environmental compartments, they might also be bioavailable to organisms present in the same surroundings. Their chemical properties like stability, lipophilicity and persistence, give them the ability to easily accumulate into fatty tissues. When pollutants are not excreted or metabolized in the body, they can be passed on from prey to predator, resulting in elevated POP concentrations in species at higher trophic levels. As humans are also at the higher trophic levels, ravelling details about bioaccumulation and biomagnification processes are of high importance for an accurate risk assessment about POPs in edible species.
In this study, POP concentrations in aquatic species collected from the Scheldt estuary are linked with environmental concentrations and the trophic level of the species, to investigate which POPs are bioaccumulated from the environment into a certain species and which are even biomagnified through the estuarine food chain.
Therefore fish, crabs, mussels, shrimps, snails, worms, plants and sediment were collected in June 2011 from three locations (Terneuzen, Bath, Antwerpen) along the Scheldt estuary by fike fishing, trawl fishing and by hand. In all the biota samples, 33 PCB congeners, 7 PBDEs, DDXs, chlordanes, HCHs and HCB were targeted for analysis. In the sediment samples, also PBDE 209 was measured. To determine the trophic position of the collected species, carbon and nitrogen stable isotope ratios (δ13C and δ15N) were measured.

ET04A-5
Food web accumulation of cyclic siloxanes in Lake Mjøsa, Norway
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The biomagnification potential of cyclic volatile methyl siloxanes (cVMS) was analysed in the Lake Mjøsa food web in Norway leading to brown trout (Salmo trutta). The food web biomagnification of octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexatetrasiloxane (D6) was analysed by trophic magnification factors (TMFs) and compared with TMFs of legacy contaminants such as polychlorinated biphenyls (PCB) and polybrominated diphenyl ether (PBDE). Of the cVMS, only D5 showed significant TMFs with values above 1, implying biomagnification. The food web accumulation of D5 was however, sensitive to species included at the higher trophic level, thus whole food web TMF differed from TMF when smelt or trout was excluded. For legacy POPs (e.g. PCB-153), the TMFs were insensitive to the food web composition, as was also reflected in a better model fit for PCBs than for D5. Nevertheless, the present study documents food web biomagnification of D5 in the Lake Mjøsa food web, from zooplankton (Daphnia and Calanus) and Mysis to planktivorous (vendace and smelt) and piscivorous (trout) fish.

ET04A-6
Trophic transfer of decamethylcyclopentasiloxane (D5) in aquatic food webs in comparison to polychlorinated biphenyls used as benchmark compounds
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The potential of a chemical substance to accumulate in aquatic organisms and to increase in concentration with increasing trophic level are criterions that are used to classify substances as being bioaccumulative in the environment. . Bioaccumulation is the accumulation of a substance in the tissues a living organism through any route. Biomagnification is the increase in concentration of a substance in living organisms that are separated by a single trophic level step on a food chain. Trophic magnification, which describes the increase in concentration of a substance in living organisms that occupy successively higher trophic levels within a food web, is used to assess bioaccumulation and biomagnification of chemicals in the environment.
Sediments and aquatic organisms were analyzed to evaluate trophic magnification of decamethylcyclopentasiloxane (D5; CAS No. 541-02-6) in the freshwater food web of Lake Pepin (Minnesota, USA) and in the marine food web of Oslofjord (Norway). Isotopic signatures for the stable isotope of nitrogen (^{15}N) and carbon (^{13}C) were used to identify trophic level positions occupied by the organisms and to evaluate the flow of carbon in the food webs. Additional samples from Lake Pepin were also analyzed for the polychlorinated biphenyl materials 2,2',4,4',5,5'-hexachloro-1,1'-biphenyl (PCB-153; CAS No. 35065-27-1) and 2,2',3,4,4',5,5'-heptachloro-1,1'-biphenyl (PCB-180; CAS No. 35065-29-3), which are 'legacy' chemicals that are both known to bioaccumulate in aquatic organisms and biomagnify in aquatic food webs. Concentrations of D5 in Lake Pepin and Oslofjord were greatest in the lowest trophic levels and significantly decreased up the food web, with the lowest concentrations being observed in the highest trophic levels. In contrast, concentrations of PCB-153 and PCB-180 in Lake Pepin were lowest in the lowest trophic levels and highest in the highest trophic levels. These results indicated that trophic dilution of D5, not biomagnification, was occurring across the aquatic food webs. The high level of agreement for results between Lake Pepin and Oslofjord demonstrated that trophic dilution of D5 was not related to differences in the environment (freshwater vs marine) or to exposure.

ET04B-1
Sources and mechanisms of cadmium bioaccumulation by the freshwater decapod crustacean Macrobrachium australiense
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Metal bioaccumulation by aquatic invertebrates in the environment can often be explained by chemical parameters such as metal concentrations within the solution and particulate phases by exposure-dose-response relationships. Simple relationships of this type are not known for invertebrates in the highly turbid Strickland River, Papua New Guinea. Here, indigenous prawns demonstrate significant differences in bioaccumulated metal concentrations (predominantly cadmium) between populations exposed to mining effluents compared to those in reference tributaries. However, metal concentrations of waters and sediments are not significantly different between sites.
This study investigated the potential sources and mechanisms of cadmium (Cd) bioaccumulation by the freshwater decapod Macrobrachium australiense using ^{109}Cd-labelled

water and food sources. Synthetic river water (SRW) was spiked with environmentally relevant concentrations of Cd and prawns were exposed for seven days with daily renewal of test solutions. Prawns were subsequently allowed to depurate in Cd-free SRW for fourteen days. Dietary assimilation of Cd was assessed through pulse-chase experiments where prawns were fed ^{109}Cd-labelled fine sediment, filamentous algae and carrion (represented by cephalothorax tissue of water-exposed prawns). Radio-analyses during the exposures were used to determine influx and efflux rate constants for Cd in water, and the assimilation efficiency (AE) and efflux rate (K_e) of Cd from each dietary source.
Results indicated that M. australiense readily uptake Cd from solution and that uptake rate increased linearly with increasing exposure concentration. During depuration, water efflux rates were low (0.9 ± 5 % d^{-1}) and were not dependent on exposure concentration. AEs of dietary sources were comparable for sediment and algae (approx 50 %), but lower for carrion (33 %) and efflux rates were low (0.2-2.6 % d^{-1}).
The results demonstrated that prawns are likely to bioaccumulate Cd readily from both water and food sources. The rapid uptake but slow efflux of bioaccumulated Cd may explain why monthly or weekly measurements of Cd in water and sediments provide inadequate information regarding exposure or dose to explain metal accumulation patterns. A biokinetic model of Cd accumulation by M. australiense is presented based on the findings.

ET04B-2
Bioavailability of dissolved organic carbon-complexed 65Cu-DOC
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Stable Cu isotope (^{65}Cu) was complexed with various representative dissolved organic carbon (DOC) types, including coastal seawater DOC, fulvic acid (FA), cyanobacteria spirulina (SP) DOC, histidine (His), cysteine (Cys), and lipophilic diethyldithiocarbamate (DDC) at different concentrations. The uptake of these dissolved Cu species by the coastal green mussel Perna viridis was quantified for the first time. Copper complexed with different DOC types were taken up in some measure by mussels, depending on the DOC types. However, complexation generally reduced Cu uptake as compared to that of inorganic Cu species, and DOC type-specific negative relationships were found between DOC levels and Cu uptake. Strong Cu binding sites (including His and organic sulfur functional groups) within DOC appeared to control the inhibitory effects of DOC on Cu uptake, possibly due to the competitive binding of Cu between the dissolved phase and biological membranes. Therefore, differences in strong Cu binding site levels may explain the differences in bioavailability of Cu complexed with different types of DOC. At the same time, the variations in Cu-DOC uptake may also be partly attributed to the absorption of Cu-DOC complexes, especially for the small Cu-DOC complexes (e.g., Cu-Cys, His or DDC). Our study highlights the importance of considering the specificity of Cu-DOC complexes when assessing biological exposure to dissolved Cu in natural waters, especially during events, such as phytoplankton bloom periods, that could modify DOC composition and concentrations.

ET04B-3
A stratified probabilistic survey of mercury in biosentinel fish and Diffusive Gradient in Thinfilm devices in San Francisco Bay, CA, USA
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Although there are many regional programs to monitor contaminants in fish, few employ probabilistic survey designs, with hypothesis testing and comparison across biosentinel types. We surveyed mercury (Hg) concentrations in forage fish to determine spatial patterns in Hg bioaccumulation across 99 sites in San Francisco Bay. We also compared these results to concentrations in field deployed Diffusive Gradient in Thinfilm devices (DGTs), and determined Hg stable isotopes in fish and sediments, to evaluate potential sources of fish Hg. Results indicated that forage fish Hg and Hg isotopes followed a broad spatial gradient in the Bay. Fish Hg concentrations and δ^{202}Hg isotopes significantly declined with distance from the Guadalupe River, which drains historic mining areas. Fish and sediment Hg isotopes were highly correlated (r = 0.91), suggesting sediments derived from mining and other historic sources to be the primary source of Hg in the fish. Fish Hg concentrations were reduced at sites draining waste water treatment plants, suggesting biodilution due to nutrient inputs. Fish and DGT Hg concentrations were not well correlated, with DGT samples exhibiting high variability among nearby sites, and elevated Hg concentrations near locally contaminated sediments. In combination, these findings indicate that Hg methylation and bioaccumulation in San Francisco Bay involves decoupled processes operating on separate spatial scales. Wheras DGTs reflect processes operating at local scales, the ultimate provenance of fish Hg appears to be historic sediments, driven by broader regional gradients.

ET04B-4
The role of feeding habits in controlling the Hg bioaccumulation and biomagnification in freshwater tilapia
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The bioaccumulation and biomagnification of mercury (Hg), especially methylmercury (MeHg), is commonly found in aquatic organisms. The mercury level in fish is not only related to its surrounding water conditions but also the feeding habit as well, and predatory fish always has high Hg level because of the Hg biomagnification through trophic transfer. Whether the biomagnification potential (in terms of trophic transfer factor, TTF) could be influenced by feeding habit is an interesting but unknown question. To explore this issue, we conducted a 40-days Hg (both inorganic and organic form) accumulation experiments, by feeding freshwater tilapia with three types of Hg labeled food (aquatic grass, freshwater shrimps, commercial pellets) at a certain ingestion rate. The results showed great differences between inorganic Hg and MeHg accumulation patterns. More important, it revealed for the first time that the prey type could greatly affect the accumulation patterns as well as fish growth and the Hg biomagnification potential. The biokinetic parameters (assimilation efficiency and efflux rate) were further determined by using radioisotope technique, and provided a good explanation of the underlying mechanism. Moreover, a three-month field study was designed to investigate the influences of feeding condition to Hg accumulation process in tilapia under local conditions, by comparing the growth and Hg accumulation of fish feeding on commercial food and natural food. These results again showed the important role of feeding habit in controlling the Hg bioaccumulation and biomagnification process in fish.

ET04B-5
Dietary accumulation, maternal transfer and metabolic inter-conversion of methoxylated, hydroxylated and synthetic PBDEs
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Polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants, and the structurally related hydroxylated PBDEs (OH-PBDEs) and methoxylated PBDEs (MeO-PBDEs) occur in precipitation, surface water and in wildlife and humans. The occurrence of OH-PBDEs in wildlife and humans is of considerable concern due to their greater toxicities relative to PBDEs and MeO-PBDEs. Although the precise origin of OH-PBDEs has not been determined the dominant hypothesis has been that OH-PBDEs are formed by hydroxylation of PBDEs by phase I enzymes and MeO-PBDEs are then formed by methylation of the OH-PBDEs. The aim of this study was to investigate the hypothesized biotransformation or inter-conversion of PBDEs, MeO-PBDEs and OH-PBDEs. Specifically, we used 1) an *in vitro* approach with hepatic microsomes from metabolically activated rats, chicken and rainbow trout to characterize the metabolic relationships among these compounds; and 2) an *in vivo* approach to study the dietary accumulation, maternal transfer, and tissue distribution of PBDEs, MeO-PBDEs and OH-PBDEs in sexually mature Japanese medaka (*Oryzias latipes*). Results obtained both from *in vitro* and *in vivo* experiments demonstrated significant production of OH-PBDEs from MeO-PBDEs while metabolization of PBDEs to OH-PBDEs was negligible. It is hypothesized that this previously unidentified mechanism is the main contributors of OH-PBDEs found in wildlife and humans. These results suggest that risk assessment of PBDEs and their metabolites needs reevaluation and human exposure to MeO-PBDEs that occur naturally in marine organisms should be considered.

ET04B-6
Use of fugacity ratio methodology for assessment of bioaccumulation potential: PCB Congeners versus cVMS materials D4 and D5
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Participants in a 2009 SETAC Pellston Conference concluded that the fugacity ratio approach is a practical framework for decision making in chemical management and regulation, particularly with regard to assessment of bioaccumulation potential. A fugacity ratio expresses the status of chemical distribution relative to a state of chemical equilibrium, which is thermodynamically defined as a situation where the fugacity ratio is 1. A fugacity ratio greater than 1 therefore indicates that the chemical in the organism is able to achieve a higher fugacity (or chemical activity) than that in the medium to which it is exposed. In that case, the organism is able to magnify the chemical potential in its environment. A fugacity ratio less than 1 implies that the chemical concentration in an organism is less than its thermodynamic equilibrium value in the

medium to which it is exposed; this is essentially the opposite of biomagnification and is termed 'biodilution'. The application of the fugacity ratio methodology has a number of advantages. First, the technique can make use of all laboratory data for which bioaccumulation metrics exist and it can also use data from field monitoring programs. Secondly, the fugacity ratios are able to express the alphabet soup of bioaccumulation measures (i.e., BCF, BMF, BSAF, TMF) on a similar basis, i.e., as a dimensionless ratio. Third, the fugacity ratios can be visualized in a weight-of-evidence approach, which allows all available data to be viewed together and which also increases confidence in the outcome of the assessment. Most importantly, the fugacity ratio approach provides a formal method for testing the hypothesis whether biomagnification occurs by testing whether fugacity ratios are generally greater than unity (1.0). This approach has been applied to the cyclic volatile methylsiloxane (cVMS) materials octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), along with positive controls, such as PCB congener 153 (2,2',4,4',5 ,5'-hexachlorobiphenyl). The laboratory fugacity ratios of PCB 153 are >>1 for fish/water (BCF), fish/food (BMF), and benthic/sediment (BSAF). Field data also show fugacity ratios for PCB 153 greater than one in biota/water, biota/sediment, and in food webs. In comparison, similar laboratory and field fugacity ratios for D4 and D5 were less than 1.0

ET04C-1

OECD TG 305 under revision: effect of different extraction procedures for the determination of aqueous analyte concentrations on the result of fish bioconcentration studies

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Bioconcentration factors (BCFs) are usually determined in fish bioconcentration studies according to OECD TG 305. The standard method for water sample preparation in BCF tests is liquid-liquid extraction (LLE) providing total analyte concentrations. Highly hydrophobic organic chemicals (HOC, with log Kow > 5) readily bind to organic matter (OM) leading to a reduced bioavailability for fish which may lead to an underestimation of the true uptake when total aqueous concentrations are measured. Solid-phase microextraction (SPME) enables the determination of total and freely dissolved aqueous concentrations. However, SPME is still a rather unusual method in regulatory ecotoxicological research. A validation approach was carried out to investigate the suitability of SPME for BCF studies.

In a first laboratory approach the effect of different types and concentrations of organic matter (Aldrich humic acid (AHA), fish feed, and filter residue taken from flow-through fish tests) on the bioavailability of highly hydrophobic organic chemicals (HOCs, with log Kow > 5) such as hexachlorobenzene, o-terphenyl PCB 153, PCB 209, and benzo(g,h,i)perylene was tested. Analysis of samples with SPME was compared to conventional sample preparation with LLE.

The bioconcentration potential of the selected HOCs was then investigated in two flow-through fish tests with rainbow trout according to OECD TG 305. Analyte concentrations in the water phase were measured after extraction by LLE and SPME. Based on the results obtained steady state and kinetic BCFs were estimated for all test items.

The results of the flow-through fish tests show that the analysis of total aqueous concentrations is leading to comparable results when LLE and SPME (with internal standards correction) are applied. However, total organic carbon (TOC) concentrations in the test water should be kept as low as possible in BCF tests on HOCs to avoid the underestimation of the true uptake when total aqueous concentrations are measured.

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ET04C-2

The effect of pH on the toxicity and bioaccumulation of weak organic acids and bases

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The effect of pharmaceuticals and veterinary drugs in the environment has recently become an area of focus. In fact, the toxicity and bioaccumulation of many of the most common compounds have been documented. However, most pharmaceuticals are acids and bases. Of a data set of over 500 pharmaceuticals, it was found that 77.5% had a group that could ionize within the biologically relevant range. Because ions are more polar than their corresponding neutral counterparts, the biological uptake of these compounds is dependent on the pK_a value of the compound, and on the pH of the water in which they are dissolved.

Recent work has revealed that the toxicity and bioaccumulation of acids and bases may be over 100 fold higher at pH values that induce the more neutral state. For acids, compounds are more toxic and bioacumulative at pH values that lie below the pK_a value of the compound while bases are more toxic and bioaccumulative at pH levels above the pK_a value of the compound. It has also been found that the pH effect is higher for those compounds with intermediate K_{OW}.

For an accurate risk assessment of these compounds it is therefore necessary to take the pH of the exposure solution into account. However, testing toxicity and bioaccumulation at multiple pH levels represents a great economic cost - particularly when it is considered that up towards 33% of the industrial chemicals registered for REACH are in fact ionizing compounds .

Based on a compilation of available pH dependent toxicity tests using ionizing compounds it is stipulated that a single worst-case test may suffice. For acids this would optimally be two pH units below the pK_a of the compound, and 2 units above for bases. For some compounds this will lead to tests outside biologically relevant range, wherefore a minimum of pH 6 and maximum of pH 9 is suggested for these tests. Acids with pK_a values below 2, and bases with pKa values above 12 do not undergo pH dependent ionization in the biologically relevant pH range, wherefore standard test procedures for these compounds can be applied. The effect of pH on the uptake of zwitterionic and amphoteric compounds has not been investigated, but it is speculated that the highest toxicity will be found at the isoelectric point.

ET04C-3

Modelling specific mechanisms of bioaccumulation: protein binding and active uptake of surfactants

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Chemicals that bioaccumulate by specific mechanisms in addition to lipid/water partitioning reveal characteristic physico-chemical properties. The range of substances showing specific (i. e. active) mechanisms of uptake in the gastro intestinal tract (GIT) or specific protein binding include, for example, poly- and perfluorinated carboxylic acids.

Data describing processes and symptoms of specific mechanisms of bioaccumulation of surfactants (target chemicals), pharmaceuticals and industrial chemicals (reference chemicals) have been retrieved from the literature. Emphasis was laid on interactions (partition coefficients, rate constants and binding affinities) related to binding to serum proteins, surface activity and active transport phenomena.

Intercorrelations of relevant properties reflect similarities and differencs between mechanisms of bioaccumulation of diverse chemical classes. Multivariate statistics are applied to discriminate the rate-limiting processes causing different bioaccumulation of surfactants, particularly perfluorinated carboxylic acids. The effects of solvent polarity will be addressed for different biophases (lipids, proteins, etc.) as well as the influence of critical micelle concentration (CMC). The known mechanisms of active uptake are specified with regard to chemical classes and organism groups. Possible dependencies between particular uptake mechanisms and intrinsic features of the chemicals are analysed.

Abraham-type modelling (LSER linear solvation energy relationship) has been applied to describe mechanisms of bioaccumulation in diverse partitioning systems, e.g. lipid/water, membrane/water, protein/water. LSER models explicitly consider the relevant contributions of hydrogenbondacceptors (α) and -donors (β) as well as effects of the molar volume of the solute relative to the respective solvent (VI/100). Calculated LSER input parameters allow for prospective modelling based on mechanistic interpretationen of relevant processes.

The modelling of specific mechanisms allows to predict the bioaccumulation potential of surfactants, e.g. poly- and perfluorinated carboxylic acids, based on process-based criteria for protein binding, surface activity and active transport phenomena. LSER modelling provides for a mechanistic interpretation of relevant processes.

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ET04C-4

Partitioning of organic chemicals to structural proteins

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Bioaccumulation of organic chemicals is typically described on the basis of the assumption that the partitioning into lipid phases is the dominant accumulation mechanism. A number of partitioning/binding studies have been reported for blood proteins such as serum albumin. However, there are only limited data available for partitioning of organic chemicals to structural proteins. These proteins are not made to bind chemicals, and thus their binding may be nonspecific, and the extent of the binding may be smaller than that of proteins that serve as a transporter or a receptor. However, relative abundance of structural proteins is generally high in organisms and thus partitioning to these proteins can be significant. In this study, binding of various neutral organic chemicals to several model structural proteins is evaluated with experimental and modeling approaches. Proteins from chicken and codfish muscle were similar in terms of binding of neutral organic chemicals. In contrast, binding by collagen was generally weaker than that of the muscle proteins. Binding to bovine serum albumin (BSA) measured before was generally stronger than that of the studied structural proteins. Thus, although serum albumin is often considered as a generic protein, our comparison suggests that the use of serum albumin as a model for structural proteins can substantially over-estimate the binding capacity of the bulk protein in organisms. To establish estimation models, a polyparameter linear free energy relationship (PP-LFER) model was calibrated using the measured partitioning data. Fitting of the PP-LFER model to these muscle proteins was substantially better than that to BSA. Finally, a composition-based

mass distribution model was used to estimate tissue-water distribution coefficients. Protein-water and lipid-water partition coefficients needed for this model were estimated using the PP-LFER models. The results suggest that the protein fraction could have little contribution to the accumulation of hydrophobic compounds even in lean tissues. In contrast, a high contribution of the protein fraction is estimated to the partitioning of some polar compounds to such lean tissues. The data and estimation models presented here will be useful to understand and estimate the accumulation capacity of organisms/tissues in varying compositions.

ET04C-5

Interaction of environmental contaminants with P-glycoprotein from trout

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P-glycoprotein (P-gp) is a membrane protein acting as cellular efflux pump of a wide variety of structurally unrelated chemical compounds. It confers "multixenobiotic resistance"(MXR) of cells and has an important role as "active barrier", preventing compounds from crossing tissue-compartment interfaces. There are two aspects of chemical interaction with P-gp: 1) By preventing chemicals from crossing compartment interfaces, such as from food into the blood-stream in the gut, bioconcentration rates are lower than would be expected from physico-chemical properties of a compound; 2) Chemical interaction with P-gp can lead to disruption of its function, thus rendering the P-gp barrier for chemicals dysfunctional. This could explain why bioconcentration of certain chemicals varies when present in combination with other compounds. There is recent evidence that P-gp from fish- in analogy to the well-studied homologs in mammals - also has properties as MXR transporter. However, test systems for functional studies of P-gp from ecotoxicologically relevant fish species are missing. We have recently established a cell line derived from RTL-W1, a rainbow trout (*Oncorhynchus mykiss*) liver cell line. The cells overexpress trout P-gp and were obtained by continuous exposure of RTL-W1 cells to low levels of vinblastine, a toxic P-gp substrate. Whereas P-gp is highly overexpressed in those cells, other efflux transporters are down-regulated compared to the wild-type cell line. Thus, these cells provide the opportunity to study specific interactions of chemicals with trout P-gp. As expected, the selected cells are substantially more resistant to the toxic cancer drug, doxorubicine, than the wildtype cells, as determined in cytotoxicity tests after 3 days of exposure to different doxorubicine concentrations. The selected cells were also more resistant to the pesticides, rotenone and metazachlor, indicating that P-gp activity may result in lower bioconcentration than would be expected. Pentachlorophenol and celestolide caused increased accumulation of the fluorescent transporter substrate calcein-am in the selected cells, indicating that those compounds disrupted P-gp activity, acting as chemosensitizers. Our selected trout cell line could be a useful component of a bio-tool kit for studies of ADME (adsorption-distribution-metabolization-excretion) processes of chemicals that are important to understand factors determining bioconcentration of chemicals in fish.

ET05 - Ecotoxicology and ecosystem services: a southern perspective

ET05-1

Regional scale risk assessment of threats to the yellowfish (Labeobarbus spp.) and the ecosystem services they provide in the in the Vaal River, South Africa's hardest working river

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The Vaal River is South Africa's most economically valuable aquatic ecosystems, and one of Africa's hardest working rivers. The ecosystem services provided by this system include a unique yellowfish dependent angling industry which is considered to be one of the largest and socially most valuable services of the system. Due to the excessive use of the Vaal River, many stressors occur which may pose a threat to the yellowfish populations of the Vaal River and the socio-economic values they offer to society. The nature and extent of threats by stressors to the yellowfishes in the Vaal River is largely unknown, and the current social and economic value of the yellowfish dependent angling industry in the Vaal River has not been documented. The aim of this study is to carry out a regional scale risk assessment of threats to the sustainability of the yellowfishes in the Vaal River and associated social and economic value of the yellowfish dependent angling industry in the system. The approach adopted to reach the aim includes the application of the relative risk model to assess threats by multiple stressors to yellowfish populations in nine risk regions in the Vaal River. Thereafter a socio-economic assessment of the value of the yellowfish dependent angling in the Vaal River has been undertaken. This information has been used to assess the threats to the yellowfish in the Vaal River and the ecosystem services they provide. Findings show that yellowfish in the Vaal River are not only of ecological importance as a part of the biodiversity, but of great social and economic value to South Africans as well. The seasonal economic value of the yellowfish dependant angling industry was determined to be worth over US$16.7 million. Thereafter, the study shows that due to the excessive use of the ecological services in the Vaal River, this value of yellowfish is at risk of being impacted on by chemical pollution, flow alterations, habitat alterations and disturbance to wildlife stressors. Although currently conditions may be acceptable, trends show that if increasing use of the ecological services of the Vaal River continue the likelihood that the biodiversity of our country would be threatened and that many South Africans would suffer social and economic losses. To avoid these losses and possibly enhance the value of yellowfish as an ecosystem service in the Vaal River existing environmental management regulations should be implemented.

ET05-2

Ecotoxicological study about the effects of Endosulfan in the viviparous fish Jenynsia multidentata (Anablepidae, Cyprinodontiformes)

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Ecosystem services such as purification of air and water are being impaired by many human activities such as unsustainable agricultural practices. The use of pesticides leads to the pollution of many aquatic habitats worldwide. Viviparous fish Jenynsia multidentata (Anablepidae, Cyprinodontiformes) is proposed as a bioindicator because its wide distribution along the Neotropical region of South America and inhabits polluted an unpolluted sites. The aim of this work was to develop an integral analysis of pesticide effects at different levels of organization in J. multidentata under Endosulfan (EDS) exposure. First, the acute toxicity (LC50) of technical-grade EDS was determined and histomorphological alterations in gills and liver was described and quantified. Second, sublethal toxicity tests (0.014-1.4 µg.L-) were carried out. As biomarkers of effect, response of detoxification and antioxidant systems (GST, GPx, GR, and CAT) and the effects on Lipid Peroxidation (LPO) were measured in different organs. Neurotoxic effects of EDS were evaluated through the AchE activity in brain and muscle. At behavioral level, swimming activity was recorded. As biomarkers of exposition, accumulation of technical-grade of EDS and Endosulfan sulfate (ES) was measured.
The LC50-96 was lower in males (0.72 µg L-1) than females (1.32 µg L-1). The histomorphological alterations in gills such as epithelial lifting are considered as defense responses to minimize the entry of EDS into the blood stream. In liver, the histological alterations varied between reversible changes at lower concentrations to irreversible changes at the highest concentration. At biochemical level, liver and brain were the most damaged organs and presented the highest levels of LP. At behavioral level, hypoactivity was observed in relation to the exposure time. These swimming activity changes were associated with the inhibition of AchE activity in muscle modifying the normal behavior of fish. The accumulation of the metabolite ES in all tissues indicate biological transformation of EDS. Moreover, measurement of technical grade EDS as well as ES is suitable biomarkers of exposure under field conditions under field conditions. If the stressful conditions persist, the mentioned alterations would lead to changes at population levels.

ET05-3

Effects of Chlorpyrifos exposure on growth and feed utilization in Australian catfish, tandanus tandanus

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The native Australian catfish Tandanus tandanus, also called the "eel-tailed catfish is found in most river systems throughout the country and in pre-settlement times was a major food fish for indigenous Australians. It is an edible species that is still favoured by rural communities. It is also considered to have potential for aquaculture. However several attempts to develop and aquaculture industry for this species have failed to date. Their wild catch declined dramatically from "fairly abundant"during 1970-1971 to the species being declared "fully protected"in most of the regions of the country at present. Organophosphate (OP) pesticides are widely used in Australia and are therefore a common contaminant in the aquatic environment. These chemicals may have significant impacts on non-target aquatic species, including fish which are particularly sensitive to OP toxicants and though their effects on fish have been widely studied, in Australia, no study has been conducted on the effects of pesticides on the Australian catfish. Our study evaluated the effects of a pulse exposure of chlorpyrifos followed by subsequent recovery in optimum conditions on the growth and feed utilization of Australian catfish, T. tandanus, simulating field pesticide exposure conditions T. tandanus were exposed to a short term pulse of chlorpyrifos at 2 or 10 µg L-1 and grown in optimal conditions for 6 weeks. Growth and feed utilization of catfish were significantly impaired after exposure to chlorpyrifos. The hepato-somatic index of catfish exposed to 10 µg L-1 chlorpyrifos was significantly elevated. The bi-weekly growth rate of exposed fish increased with time post-exposure and was correlated with the recovery of brain Acetylcholinesterase (AChE) activity. The results revealed that the fish were able to recover from the pulse of pesticide exposure since their growth rate was found to be as high as the control during the last 2 weeks. It is therefore possible, that poor growth performance resulting from exposure to OPs would be compensated for and fish recover if favorable conditions are prolonged. This implies the necessity for the reduction of repeated spraying of OP pesticides in the field and it is recommended that more such investigations be conducted on native, iconic food fish of cultural value.

ET05-4

Screening level ecological risk assessment of rewetted acid sulfate soils in the lower Murray River System, South Australia

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The River Murray, adjacent wetlands and the Lower Lakes (Alexandrina and Albert) in South Australia have been classified as wetlands of international importance under the Ramsar Convention, due to their unique ecological and hydrological significance. Prolonged drought due to the southward movement of the subtropical ridge atmospheric pressure system as a direct result of climate change, compounded by over allocation of water upstream, has resulted in very low water flows from 2005-2010. Consequently, the Lower Murray River system was being seriously impacted by a combination of low water levels and the presence of acid sulfate soils (ASS).
If detected and managed appropriately, ASS may not pose a major risk, however, if disturbed or left unmanaged, they pose risks to humans and the environment. To better understand the risks associated with rewetting of these ASS, and to provide recommendations for appropriate management options, a screening level ecological risk assessment was undertaken. The aim was to protect ecosystem services from the risks of ASS that may alter a balanced community of aquatic biota.
Risks to aquatic biota from stressors including metals, acidity, major ions and nutrients released from Murray River system dried soils that had been rewetted via rainwater or river water were determined. Predicted environmental concentrations, with correction for background water quality data, were calculated from metal release data for the soils in laboratory leachate experiments for current water levels (about -0.5 AHD) for four geographical areas using a range of exposure scenarios. These were compared to predicted no effect concentrations derived from hardness-corrected water quality guidelines, both acute and chronic. An overall risk ranking for each stressor at each location was determined, based on the likelihood and consequence of effects. This SERA provided the first semi-quantitative assessment of risks to aquatic biota associated with rewetting of ASS following climate change-induced drought.

ET05-5

Anthropogenic impacts on ecosystem services of a subtropical lake in southern Africa

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Lake Pongolapoort (LP), situated in the sub-tropical northern KwaZulu-Natal, South Africa (SA), is the recreation angling hotspots of SA and one of the few places in SA to catch Africa's most sought after freshwater angling species, the tigerfish, Hydrocynus vittatus. Due to the popularity of angling, tigerfish have a direct positive impact on the socio-economic wellbeing of the local community. As tigerfish in SA is listed as a threatened species, legislation requires that captured fish are released alive, (catch-and-release angling, C&R), however, the ultimate success of C&R angling depends on high release survival rates. The potential thus exist that, although the angling industry is a main ecosystem service of the LP, it might also be a major threat to the tigerfish population. Furthermore, the surrounding area of the LP is an endemic malaria area. The application of DDT in this area as a malaria-control measure has been ongoing since 1946. As relative high levels of DDT has been detected in LP tigerfish muscle, this might further impact on the health of LP tigerfish. The aim of this study was thus to quantify two of the most threatening anthropogenic impacts (recreational angling stress and DDT) on the tigerfish population of LP. A total of 109 H. vittatus were sampled in February 2009 (n=62), July 2009 (n=19) and April 2010 (n=28). Fish were collected by standard recreational angling and time to land and handle the fish were recorded. Fish were then anaesthetised in a 32 mg·"-1 concentration of clove oil, 2m" blood was drawn from the caudal vein and analysis for plasma glucose, lactate and cortisol concentrations. Axial muscle samples of 30 (February 2009) and 15 (July 2009) tigerfish were removed and analysised for DTT. A one-way ANOVA revealed that the plasma cortisol and plasma glucose concentrations were not significantly influenced by angling time (cortisol P = 0.109; glucose P = 0.887). However, there was a strong positive correlation (r2 = 0.749) with an increase in total time causing an increase in lactate concentrations. Levels of DDT and metabolites were found in all the tigerfish muscle samples. A high DDE/DDT ratio indicated historical input of DDT into the system. The data presented here as well as additional data on tigerfish age and histopathology show that the tigerfish population of LP is under stress due to anthropogenic stressors, such as recreational angling stress and historic DDT application as a malarial control maganism.

ET05-6

The impact of gold mining on mercury pollution in the Witwatersrand Basin, South Africa

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Gold mining in the Witwatersrand basin, SA contains the biggest and richest mines in the world. Mercury, which occurs in some gold-bearing ores, was also used for gold recoveries till 1915 and is still used in artisanal mining. Presently some old gold tailings dumps are reprocessed. Consequences of these activities are the release of Hg to the environment.
Risk assessment of Hg pollution in SA is based on total concentrations which is not sufficient to understand its transport and fate. It is necessary to evaluate Hg speciation, and characterize potential sources, pathways, receptors and sinks in order to implement mitigation strategies and minimize risk.
The purpose of this work was to carry out an assessment of Hg pollution in the Wesr Rand areas with long history of gold mining and to investigate the Hg distribution, transport and fate.
Water, soil and sediment samples were collected during the late dry season in mining site located on the West Rand and through the game reserve to the Cradle of Humankind. Samples were prepared using Rpodrigez Martin-Doimeadios et al. method [2], and Hg species, inorganic (IHg) and methylmercury (MHg), were determined by Isotope Dilution GC-ICP-MS validated with certified reference material (IAEA405).
High Hg concentrations were found in surface water (0.01 - 220.63 ng IHg L-1 and 0.04 - 2.12 ng MHg L-1) and sediments (46 - 2090 ng IHg g-1 and 2 - 5 ng MHg g-1) (Fig.1). Mercury enrichment was observed in bulk sediment reaching 3900 ng IHg g-1 and 83 ng MHg g-1 at 20 cm depth.
Field parameters measurements in water samples have demonstrated a typical case of acid mine drainage with high redox potential, high electrical conductivity and the pH dropping as low as 3.
High Hg concentrations found in this study suggest that post-gold-mining operations may be important contributors to the pollution of stream draining the mining site and affect the nearby game reserve and precious Cradle of Humankind. Mercury enrichment in bulk sediments also confirms the long term pollution of the site. Organic matter and sulfur contents together with redox potential and pH appear to be important contributing factors to the methylation process. Methylation of Hg is occuring in the water system and allow MHg to enter the food web.

ET06 - Ecotoxicology of amphibian and reptiles. Novel approaches for linking contaminant effects with population declines

ET06A-1

Changes in morphology, behaviour and energy reserves of the African Toad Bufo regularis exposed to lethal and sublethal concentrations of Diazinon

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The lethal and sublethal toxicity of the insecticide diazinon on the African toad, Bufo regularis were evaluated to assess changes in morphology, behaviour and energy reserves. The median lethal concentration of the insecticide was found to be 0.44mg/l indicating the high toxicity of the insecticide. Uptake of the pesticide by the toads caused dose-dependent deformities and behavioural abnormalities. More pronounced poisoning symptoms were observed at higher concentrations. The pesticide caused differential increase in glucose levels in the blood with a concomitant reduction in liver glycogen. The increase in glucose levels and the reduction in glycogen levels indicate disorders in carbohydrate metabolism due to pesticide induced stress. The findings revealed that changes in morphology, behaviour, glucose and glycogen levels could be considered suitable biomarkers in evaluating the effect of diazinon.

ET06A-2

Combined effect of malathion and nitrate on survivability of tadpoles of Indian cricket frog fejervarya limnocharis

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We have examined individual and combined effect of realistic concentration of Malathion and nitrate on the survivability of Indian Cricket Frog tadpoles. In this fully factorial experiment, tadpoles were subjected to individual and combinations of 0, 500, 1000 and 2000 µg malathion/Land 0, 4 and 8 mg NO3-N/L. When tested alone, malathion found to be detrimental to tadpoles and reduces the survivability, while nitrate increase the survivability. The response exhibited at the combined treatment does not follow the trend recorded at different concentration of each of the tested chemical. Presence of nitrate enhances the effect of Malathion at lower concentration, while at combination of higher concentrations of nitrate and Malathion, the trend of survivability was determined by Malathion alone. Since the breeding season and tadpole stage of this frog coincide with application of pesticide and nitrate, there is every possibility that these combinations could affect frog population in agro-ecosystems.

ET06A-3

Amphibians at risk ! Susceptibility of terrestrial amphibian life stages to pesticides

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The 'global amphibian decline' is manifested with more than 30% of all amphibian species on some level of threat according to the IUCN. Pesticides are considered one factor for the reduction of species and observed population declines. Pesticides reach aquatic habitats by drift and runoff whereas they are applied at high field rates in crops where they adhere to soil particles and plants. Although amphibians are depending on water bodies for their development the majority of their adult life cycle is spent in the terrestrial habitat. So far no specific risk assessment is carried out for the terrestrial life stage of amphibians since it is assumed that they are covered by the procedures in place for birds and mammals.
We present data for presence of amphibians in two different agricultural landscapes in Germany and discuss the difference in skin properties and uptake between amphibians and mammals. An extensive review (Brühl et al. 2011) revealed that only few toxicological data exist for amphibians that were conducted to mimic an overspray scenario and dermal exposure of amphibians.
This research gap was filled by conducting rate-response test with different pesticides and juvenile Common Frogs *(Rana temporaria)* using a spraying chamber and realistic field rate application scenarios. Observed mortalities at field rates were situated between 20 and 100%. There was no difference between fungicides, insecticides and herbicides. Furthermore it remains unclear if the observed toxicity is based on the active substances or the solvents in the formulations or a combination of both.
We conclude that terrestrial life stages of amphibians are present in agricultural landscapes and crops during application of pesticides. Passage of pesticides through a permeable skin is much higher than for mammals and therefore the dermal uptake route is the most likely exposure route for amphibians. So far this is not considered in bird and mammalian risk assessment where the focus lies on oral uptake.
Our data set for the toxicity of a few registered pesticides reveals alarming mortality levels at the field rate. Therefore current risk assessment procedures are not protecting amphibians sufficiently. Hence carefully designed studies are urgently needed and it is imperative to develop a specific risk assessment procedure for amphibians to avoid further impacts on amphibian populations associated with agricultural areas.

ET06A-4
Ranking ecological risk of multiple stressors on amphibians
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In this study we rank the potential threats to amphibians in their actual and potential habitats in the Netherlands. The study combines laboratory toxic effect data (acute median Lethal Concentrations, LC50) for anuran species with environmental monitoring data for different stressors and actual survey data of anurans in the Dutch freshwater bodies. Combining all the data together, the ecological risk (ER) was calculated for ammonium, nitrate, cadmium, copper, pesticides (18 different compounds), and acidification (pH) as a probability that anuran species are exposed in the field to stressor concentrations exceeding their LC50s. The total ER at the LC50 level, as calculated for Dutch freshwaters was found to be 2.73%. We ranked the stressors in the decreasing order of their ER: pH, copper, diazinon, ammonium, and endosulfan. Taking into account the bioavailability of copper, the ER for it might be reduced. Therefore, the main threats affecting anurans populations arise from acidification of their habitats, ammonium, and certain pesticides exposures. These results are valuable for conservation management of amphibians when prioritization for mitigation of stressors is needed. The method of deriving the ER is a useful tool for location-specific risk assessment of multiple stressors for selected amphibian species and can be applied in other locations for species of concern.

ET06A-5
Are standard avian risk assessments appropriate tools addressing the risk to reptiles?
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According to the new data requirements under the current EU regulation 1107/2009, 'available and relevant data, including data from the open literature for the active substance of concern, regarding the potential effects of an active substance to birds, mammals, reptiles and amphibians should be presented and taken in to account in the risk assessment.'
Whereas for birds, mammals and amphibians specific data requirements are available, nothing is stipulated for reptiles. As stated in the Guidance of EFSA - Risk Assessment for Birds and Mammals (2010): 'information should be provided to enable an assessment of the direct impact on birds and mammals likely to be exposed to the active substance, plant protection product and/or its metabolites. In order to determine the risk, toxicity data are taken, along with an estimate of the likely exposure concentrations'. The guidance provides a tiered approach to assess both, direct acute and reproductive risk to birds. Therefore one approach for reptile risk assessment may be the most recent state of the art risk assessment applied for birds, taxonomically related to reptiles. The necessary backgrounds for such an approach, however, are reliable data on the exposure and toxicity of PPP. For reptiles such data and generic data recorded in agricultural landscapes are nearly completely missing. For that reason publically available literature was searched in order to obtain information on the ecology of reptiles in agricultural areas in North and Central Europe, relevant exposure routes, and any toxicity data of PPP may affect reptiles. The results were used to evaluate and discuss the viability of the avian risk assessment approach for reptiles. Furthermore it shows potential data gaps that need to be fulfilled to successfully implement reptile risk assessments.

ET06A-6
Composition of the herpetofauna in intensively managed monocultures in Spain
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According to the new data requirements under the EU regulation 1107/2009 reptiles and amphibians have to be addressed in risk assessments of plant protection products (PPP). But little information is available about the herpetofauna in agricultural landscapes, especially in intensively managed monocultures. However, it is important to know which reptile species occur in relevant densities inside agricultural habitats. During 2007-2009 orchards, field crops and vineyards in different parts of Spain were visited from early spring- to mid summer, and reptiles and amphibians were recorded. Overall 4 amphibians and 14 reptile species were detected. Mentionable differences were observed between the different survey areas and crops (from 6 species in fruit orchards up to 13 species in citrus orchards). The species found were classified in four different diet guilds (herbivorous, omnivorous, insectivorous and carnivorous). None of the detected amphibian and reptile species is exclusively herbivorous. All of the detected amphibians and the most lizards can be assigned to the insectivorous guild. Only the Mediterranean turtle (*Mauremys leprosa*) and the occellated lizard, *Timon lepidus*, have to be treated as omnivorous. All of the snake species feed on vertebrates. Available habitat data given in the IUCN Red List (IUCN 2011) and by Fryday & Thompson (2009) was compared to our findings per species. The grass snake, *Natrix natrix*, was the only species that might be at risk of exposure by PPPs according to Fryday & Thompson (2009), and found during our surveys. Because of the obvious differences between published data and our observations further fieldstudies are needed to obtain quantitative data on species compositions of the herpetofauna and densities of possible focal species for risk assessments for PPP in agricultural land in Spain.

ET06B-1
Thermal tolerance of amphibians and their invasive predator in a polluted environment
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It is clear that many threats such as habitat loss, chemical pollution and global warming are contributing to both local and global amphibian declines. Yet aside from a few examples, we still know very little about the role and magnitude of synergistic effects among these stressors. Demonstrated in marine ectotherms, thermal tolerance is limited by the incapability of an organism's circulatory and ventilation systems to supply sufficient oxygen to match with its oxygen demand (i.e. aerobic scope). Beyond certain critical temperatures, survival of aquatic ectotherms becomes passive and time-limited, relying heavily on anaerobic metabolism and molecular protection (e.g., induction of heat shock proteins). Detoxification is known to be an energy-demanding process. It is thus likely that an organism under thermal stress will suffer further decrease in aerobic scope and loss of performance in a contaminated environment, which may in turn threaten their survival. Our study thus focused on the relationships between pollution and thermal stress on three lowland wetland amphibian larvae in South China, namely Asian common toad (Bufonidae: *Duttaphrynus melanostictus*), brown tree frog (Rhacophoridae: *Polypedates megacephalus*) and marbled pigmy frog (Microhylidae: *Microhyla pulchra*), and the relative performance of the invasive fish predator *Gambusia affinis* as opposed to local larval amphibians. This study determined and compared lethal concentrations of two commonly used agricultural pesticides, glyphosate and methomyl, on the target amphibian and fish species, as well as lethal temperatures of these animals. The investigation on the synergistic effects of thermal stress and pesticides is currently being conducted using integrated measurements of physiological end-points and biomarkers including growth rate, oxygen consumption rate, and expression of heat shock protein and lactate dehydrogenase. Our preliminary results showed that pesticide tolerance varied highly between the three amphibian species, with *D. melanostictus* being the most tolerant, followed by *P. megacephalus* and *M. pulchra*, and that the invasive predator *G. affinis* had a wider thermal tolerance window (37.16°C) than *P. megacephalus* (33.97°C) and *M. pulchra* (34.36°C). Further information generated from this study on the synergy between pollution and thermal stress will aid in developing conservation measures to better protect amphibian populations against these rising threats in South China region.

ET06B-2
Effects of a mixture of agricultural pesticides and estrogenic compounds on developing tadpoles

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Acute and chronic toxicity tests with the Australian striped marsh frog (Limnodynastes peronii) and the cane toad (Rhinella marina) were undertaken to determine if the combination of common agricultural pesticides (glyphosate, atrazine, endosulfan) and estrogenic compounds (4-nonylphenol and estradiol) at environmentally realistic concentrations effect tadpole development and survival. There is a growing prevalence of these combinations occurring in agricultural landscapes through the application of biosolids as fertilizer. Findings indicated that complex mixtures reduced survival, and impaired growth and development compared to the individual chemical exposures. The presence of estradiol appeared to have little influence on these endpoints. Endosulfan was found to be the most toxic compound in our mixtures, causing erratic swimming behaviour and darkening of the skin following exposure to this compound alone and in mixture. However, the acute toxicity was only enhanced with the presence of sub-lethal concentrations of the other compounds. Further research will help identify specific mixtures and chemical ratios causing effects, but our results support the hypothesis that complex mixtures at sub-lethal concentrations cause detrimental effects on developing tadpoles.

ET06B-3
Effect of the acute exposure (96 h) to xenoestrogens on 'Lithobates catesbeianus' tadpoles
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The aim of this study was to evaluate if a concentration of 17-alpha-ethinylestradiol (EE2) normally found in Brazilian industrial effluents can exert some impact on the cardiac function of bullfrog tadpoles (25 Gosner stage), *Lithobates catesbeianus*. To this end, animals were exposed for 96 h to 10 nM of EE2 and their responses were compared to those obtained to controls. During exposure, the animals´ activity level (AL -% of active individuals) was monitored twice a day. Immediately after exposure, the in loco heart rate (fH - bpm) was determined. Afterwards, animals were sacrificed and the ventricles were removed for the preparation of ventricular strips to record isometric contractions (Fc - mN. mm^{-2}). The results indicated that EE2 did not affect tadpoles´ AL ($P > 0.05$), although it resulted in a tachycardia in animals exposed to EE2 (fH = 66 bpm) when compared to their controls (f_H = 52 bpm). These findings suggest that EE2 may act directly on the cardiac muscle of bullfrog tadpoles, rather than its effects being a result of an increased cardiac demand due to a higher activity level in order to the aversive stimulus of the exposure to the xenoestrogen (i.e., avoidance response). Additionally, the comparison between the values of Fc between the two experimental groups showed that the xenoestrogen exerted a positive inotropic response. Thus,it can be suggested that the increase on cardiac performance induced by the exposure of bullfrog tadpoles to this xenoestrogen elevates considerably the animal energy expenditure, diverting a large amount of energy that tadpoles could use for their growth and development. These alterations make the animals more susceptible to predators and also reduce the likelihood that they will reach reproductive stage.

ET06B-4
Novel methods to assess health of amphibian populations: preliminary results
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It is now widely accepted that amphibians are undergoing precipitous decline on a worldwide scale. In some cases reasons for decline are known (e.g. habitat loss, chytridiomysis infection, invasive species), however, specific causes in the majority of cases are not well understood. An important consideration in the study of populations is to focus on those that are in decline in order to assess causative factors. However, it is also unethical to harm individuals from these populations, and therefore there is a need to develop non-destructive methods to assess population health. We identified two sites with differing levels of pesticides (NP = non-polluted; P = polluted; characterised by analytical chemistry), and measured various endpoints related to "health"and "reproduction"of the common toad, Bufo bufo. We also sacrificed a subsample of individuals in order to correlate non-destructive biomarkers with more traditional markers of effect (blood hormone levels and gonadal histopathology). We found that weight and snout-vent length were lower in individuals from the polluted site (n = 32, t-test $p < 0.001$). Interestingly, corticosterone levels were also higher in the P group (n = 20, t-test $p = 0.07$), and a positive correlation of weight and corticosterone levels was observed in the P (n = 10, r2 = 0.43, $p = 0.04$) but not the NP (n = 10, r2 = 0.06, $p = 0.5$) site. In addition, the fat body weight (FBW) was decreased in the P ($p = 0.09$), however, weight was not correlated with FBW (n = 20, r2 = 0.01, $p = 0.15$). Neither chytridomysis nor Ranavirus were detected in any individuals. Contrary to a previous report in Bufo marinus, there was no difference in the sexually differentiated forelimb width nor nuptial pad size according to pollutant input. There was also no difference in relative testis size, however, relative Bidders' organ size was larger in individuals from the P site (n = 20, $p = 0.06$). Analysis of the testicular histopathology is ongoing. In addition, no difference in sperm parameters or testosterone levels were observed, which were also not related to any morphological androgen sensitive endpoints (forelimb width, nuptial pad size, testis size). Preliminary results indicate that survival parameters, such as size and "stress"may be affected by agricultural input, however, minimal effects on reproductive parameters were observed. The increased Bidders' organ size may be due to the higher levels of estrogens at the P compared to the NP site.

ET06B-5
Screening breeding ponds of the Common toad (Bufo bufo) in the UK for evidence of endocrine disrupting activity
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To date, there has been no attempt to assess the potential impacts of endocrine disrupting contaminants of the aquatic environment on wild populations of native amphibians in the British Isles, though this group has suffered declines in the agricultural landscape. We selected nine breeding sites of the Common toad (*Bufo bufo*) in the UK on the basis of predicted surface concentrations of agrochemicals and past population trajectories based on counts of adult toads in the breeding migration. Samples of spawn were enclosed in mesh cages, and hatching, survival, growth and development were monitored though larval development. Larvae and toadlets were sampled at several timepoints for gonadal histolopathology at five of the study sites. We also deployed semi-permeable membrane devices (SPMD) and Polar Organic Chemical Integrated Samplers (POCIS) at all sites for 4 weeks and screened extracts for endocrine disrupting activity in recombinant Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS), and in primary monolayer cultures of *Xenopus* hepatocytes for estrogen-dependent induction of vitellogenin. None of the SPMD extracts exhibited estrogen receptor or androgen receptor agonist activity. SPMD extracts from many study sites displayed weak anti-androgenic and anti-estrogenic activity in the YAS and YES, respectively, though similar activity was observed in control membranes. Among POCIS extracts, two sites exhibited concentration-dependent androgenic activity in the YAS, while three other sites (two in areas of intense arable cropping) exhibited significant estrogenic activity in the YES. Extracts from the same three sites also exhibited estrogenic activity in the *Xenopus* hepatocyte assay. Hatching rate varied among sites, but could not be fully explained by predicted surface water concentrations of pesticides, conductivity, pH or endocrine activity of the extracts. Overall survival/persistence of *Bufo* larvae in the cages was poor, which could reflect poor ability to feed, predation, or escape from the mesh cages. The sites with lowest larval survival had medium-to-low total predicted surface water concentrations of agrochemicals. Most of the sampled larvae had undifferentiated gonads, as did many of the toadlets. There were no significant differences in sex ratio among the study sites sampled for histopathology, at any of the sampling points, and there was no evidence of regressed or matured testicular oocytes in any of the sampled toadlets.

ET06B-6
Investigating the threat from pollution to the endangered species Caretta caretta (Linnaeus, 1758) in the Mediterranean: the use of novel non-invasive biomarkers
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The aim of this study was to develop and apply a non-invasive protocol, integrating biomarker responses and a contaminant analysis, in order to investigate the threat from pollution to C. caretta of the Mediterranean. For the study, forty-five specimens of this endangered species were sampled in a non-invasive way in Italian Italian marine rescue centers or free-ranging along the Spanish coasts (Murcia region). The specific goals were to: a) develop a non-destructive sampling methodology of various biological materials; b) develop and apply a set of ecotoxicological biomarkers in order to explore different levels of interaction between contaminants and the organism, from biomarkers of exposure (induction of CYP1A in skin biopsies, accumulation and excretion of porphyrin) to biomarkers of indirect and direct effects by investigating neurotoxic (esterases inhibition in plasma) and estrogenic (vitellogenin in plasma) effects, oxidative stress (lipid peroxidation in plasma), genotoxicity (Comet, diffusion and ENA assays in blood) and liver damage (ALT, AST and y-GT in plasma); c) investigate levels of contaminants mostly present in the Mediterranean (OCs and PAHs in blood; Pb, Cd, Hg in carapax); and finally, d) to elaborate biomarker and contaminant responses taking into consideration age and sampling site. The non-invasive sampling methodology was successfully developed and applied. Appropriate quantities of blood, skin biopsy, carapax and excreta were collected, for the successful development and application of biomarker and contaminant detection methods. A statistically significant correlation was found between cadmium and y-GT levels. Induction of skin CYP1A, as well as high comet assay response and total PAH levels were found in two young specimens sampled off-shore of the Calabrian coasts (Brancaleone) after an oil spill. Analysis of the results by classes of age showed that levels of PAHs and Hg tend to increase with age. The same trend was found for lipid peroxidation and ENA assay responses. The analysis of results by sampling areas showed that BChE had significantly lower levels of activity in specimen samples along the Murcia region coasts, an area of intense agricultural activities. We believe that the findings of this study contribute significantly to developing non-invasive protocols to study the threat to C. caretta from

pollution and to define appropriate actions to mitigate threats and thus to protect this species.

ET07 - Environmental OMICs: a global answer to environmental questions

ET07A-1

Environmental proteomics to predict the effects of global warming on aquatic organisms exposed to pollutants

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Beyond shifts in species distribution, most organisms are forced to acclimate or adapt to long-term changes in temperature and frequently exhibit temperature compensation mechanisms. Moreover, aquatic species are also subjected to polluted environments. It is consequently necessary to assess the combined effects of heat stress and xenobiotic exposure for predicting the impacts of global warming on wildlife. We report here the results from two studies on the proteomic responses in fish species under combined heat stress and pollutant exposure. The first study aimed at identifying proteins whose expression has been modified by temperature and selenium (Se) exposure in larval green (GS) and white sturgeons (WS). The aim of the second study was to investigate whether acclimation to 3 temperatures may lower or enhance the ability of European bullhead to tolerate subsequent exposure to cadmium (Cd). In GS and WS, gel replicates were first grouped according to heat treatment. Proteins involved in protein folding, protein synthesis, protein degradation, ATP supply and structural proteins changed in abundance in response to heat and/or Se. In bullhead, thermal acclimation was the first parameter affecting the protein expression profile, while fish's ability to respond to Cd was clearly affected during acclimation to 21°C. The identified differentially expressed proteins were associated for instance with protein turnover, folding and chaperoning, transmembrane transport, metabolic regulation, cell signalling, and cytoskeletal reorganization. This work provides insights into the interactive effects between heat acclimation and a subsequent exposure to Cd, and suggests that further studies on the identified proteins could offer essential information to better understand the mechanisms of action shared by these two environmental stresses. In conclusion, we showed using two distinct experiments that proteomics is a suitable tool to investigate the effects of heat stress on the cellular phenotype. A specific proteome could be defined for each stress condition, and likely represents the cellular responses to specific mode of action. When cross-tolerance is observed, those proteomic signatures can bring clues to understand shared mode of action. The opportunity to focus on this cellular phenotype acquired during acclimation is discussed in the perspective of evolution theories and should be taken into account in order to predict the effects of global warming on wildlife.

ET07A-2

The Concentration-Response Concept in Proteomics: effects of a narcotic (Phenanthrene) and a specific acting Substance (Gemfibrozil) to the Proteome of Zebrafish Embryos (Danio rerio)

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Proteomics studies in ecotoxicology have the potential for the detection and quantification of proteins related to chemical stress. These proteins might serve as novel biomarkers and provide insights into mode of action or mechanisms of toxicity. As studies based on 2-DE are comparatively elaborate, time-consuming and expensive, the finding of robust biomarkers is limited by the numbers of replicates, statistical methods and also by the experimental design. For these reasons, many published proteomics studies in the area of (eco-) toxicology are confined to one or two tested concentrations. As reactions at the molecular level are expected to be a function of exposure concentration, the analysis of few concentrations might direct the experimenter to fragmentary or incomplete conclusions. In the present study an experimental design for a proteomics study was tested for detection of induced protein regulation. This design included the analysis of concentration dependence without increasing the replicate number of 12 parallel 2D-gels. The tests were done using the zebrafish embryo as a vertebrate model of teratogenicity. To test whether the proteome analysis is able to discriminate between substances with different mode of action (MoA), the substances Phenanthrene (narcotic MoA) and Gemfibrozil (specific MoA) were selected for the proteomic tests. For the 2D-Gelelectrophoresis experiments, Danio rerio embryos were exposed to six different concentrations ranging from 1% of LC50 to the LC20 (Gündel et al. 2011). The selected concentrations were based upon a teratogenicity assay with zebrafish embryos accomplished in closed glass vessels. Proteomics analyses were done by 2D-DIGE technique (Marouga et al. 2005) with minimal CyDye labeling. Protein abundance profiles of around 700 signals were studied in the Phenanthrene exposure tests. About one third of the protein spots could be detected to show reactions correlating with stressor concentration. From this group about 70 protein spots already showed changes beginning at low and very low effect concentrations. In this study it could be shown that increasing the number of measured concentrations instead of replicate numbers in 2D-Gelelectrophoresis experiments, the detection of robust stress markers was possible. From these proteins many may be already detectable at very low lethal effect concentrations such as the LC01. In this way the possible output of proteomics experiments may be increased considerably.

ET07A-3

Integrative environmental genomics of cod (Gadus morhua) reveal the mechanisms underlying MeHg- and PCB 153 induced toxicity

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Atlantic cod (*Gadus morhua*) is an important species in both North-Atlantic fisheries and aquaculture. However, coastal cod populations are highly stationary, and are therefore particularly susceptible to environmental insults. Coastal- and petroleum industry are expanding into cod habitats, and limited information exist on the effects on cod to both acute- and long-term exposure to several environmental contaminants. Importantly, in order to monitor and maintain sustainable coastal cod populations, it is necessary to understand how such contaminants may affect growth, reproduction, and health of this species. Methylmercury (MeHg) and PCB 153 have several properties in common. Both compounds act as neurotoxins, they are persistent environmental pollutants, and ubiquitous contaminants that are biomagnified in aquatic food chains. To develop a deeper understanding of transcriptional and translational responses of the cod genome to MeHg and PCB 153, we have initiated a toxicogenomic approach combining transcriptomics, proteomics, and bioinformatics, as an attempt to integrate these responses into mechanistic insights. The liver proteomes of MeHg- and PCB 153 exposed cod have been resolved with both gel-based and LC-MS/MS based methodologies, while the corresponding liver transcriptome from these samples have been analyzed with microarray analysis using a cod-specific array. We have simultaneously established an in-vitro exposure system using cod liver slices for accompanying the in-vivo exposure experiments. Moreover, this interdisciplinary study has provided detailed information regarding the modes of action of both MeHg and PCB 153, including responses in several genes previously not known to be affected by these compounds. The differentially regulated proteins are also candidates for new and more sensitive biomarkers for MeHg- and PCB 153-exposure for use in environmental monitoring and risk assessment.

The project iCod: integrative environmental genomics of cod (Gadus morhua) is funded by the Norwegian Research Council (project 192441/I30). Thanks to the Genofisk Consortium and the Cod Genome Sequencing Project Team for sharing data in advance of the public release of the cod genome data (www.codgenome.no).<br type='_moz' />

ET07A-4

In vitro exposure of nanoparticles in mouse: an integrated proteomic and lipidomic study

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"Omics"is a rapidly expanding technology in both industry and academia. Its development can provide a powerful tool to promptly detect complex biological problems caused by a multitude of stressors, and contribute to the comprehension of eventual unknown mechanisms of toxicity.
Nanoparticles (NPs) are emergent compounds characterized by fast-growing research due to their potential applications in a myriad of areas as diverse as electronics, cosmetics, pharmaceuticals, environmental devices.
Numerous products NP-based are already available commercially although contradictories effects have been shown from the recent scientific studies.
The aim of this project was to elucidate the possible biological effects on primary cultures of mouse hepatocytes exposed to inorganic ($Ti0_2$, CuO, ZnO and Ag) NPs.
Briefly, the nanoparticles were characterized by scanning electron microscopy (SEM) and Z-potential analyses. The cell cultures of mouse hepatocytes were exposed to the selected NPs at 1 and 5ppm concentrations for 48 hours.
Reactive oxygen species (ROS) production was measured by cell-free dichlorofluoroscein diacetate (DCFH) assay and the treated/untreated hepatocytes were analysed and compared by proteomics and lipidomics.
The cytosolic proteome was analysed by two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) and Coomassie staining. The differentially expressed proteins were characterized by image analysis, and identified by mass spectrometry.
Lipids were separated by one-dimensional thin layer chromatography and the major phospholipids and neutral lipids were quantified by optical densitometry.
The combining of proteomics and lipidomics presented in our study is a novel approach for the detection of the impact of NPs in biological system. Although proteomics would inform about the protein expression, biological processes such as signal transduction pathway, receptor recognition, membrane protein translocation would be seriously affected by modification in the lipid composition.

ET07A-5

Altered protein expression in stickleback (Gasterosteus aculeatus) gills after silver nanoparticle exposure

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Engineered nanoparticles (ENPs) are rapidly becoming an important part of new technology and are today used in a variety of products, from computers to pharmaceuticals and body care products. Silver nanoparticles (AgNP) are the most widely used nanoparticle, especially due to its antimicrobial properties. AgNP may be introduced into the aquatic environments during production processes and from hundreds of products already present in the market. There are numerous potential environmental risks of AgNP that needs to be elucidated including its toxicity. Silver ions are toxic to fish due to their ability to bind to thiol groups inhibiting enzymes such as Na+/K+ ATPases that are extremely important to the gills osmoregulatory function. In order to study the effects of AgNP in fish, sticklebacks (Gasterosteus aculeatus) were exposed to AgNP (0,1 mg/L) and silver ions (0,01 mg/L) in a freshwater semi-static system for seven days. Gills samples were taken for proteomic studies, transmission electron microscopy (TEM) and Na+/K+ ATPase activity measurements. The behavioral (e.g. agglomeration rate) of the AgNP in the test water and levels of ionic and total silver were monitored throughout the experiment. Amine-reactive TMT Isobaric Mass Tagging was used to quantitatively label proteins extracted from gills for identification and analysis by mass spectrometry (TMT sixplexTM label reagent set, Thermo Scientific). Proteins were identified with the help of a Stickleback specific proteome database. Ionic silver exposure resulted in more regulated proteins compared to the AgNP exposure. 116 proteins were regulated in the Ag+ exposed gills (53 down and 62 up) while only 43 were regulated in the AgNP exposed (22 down and 21 up). Among the regulated proteins only nine proteins were equally affected by both treatments meaning that 34 of the observed protein regulations were AgNP specific. Several of the regulated proteins in the AgNP exposed fish have a function in cell structure and cytoskeleton. Also, a mucus forming glycoprotein (mucin 2) was up-regulated in the AgNP exposed fish gills suggesting that the gill epithelial cells were stressed. The presence of AgNP in gills cells was detected using TEM. This study was supported by FORMAS and proteomic analysis performed in collaboration with the proteomics core facility at Gothenburg University.

ET07A-6

Transcriptomic responses in Japanese medaka (Oryzias latipes) exposed to individuals or mixtures of polycyclic aromatic hydrocarbons: mixture toxicities or fasting effects?

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In the present study, we assessed the effects of single or mixtures of polycyclic aromatic hydrocarbons (PAHs) by transcriptome analysis. Four-week-old Japanese medaka (*Oryzias latipes*) were exposed to phenanthrene (5, 50 ppb), pyrene (3, 30 ppb), or their mixtures for 96 hours. Fish were sampled at 0, 24, or 96 hours after the exposure test started. Total RNA extracted from whole body of medaka was subjected to microarray experiment. Principal component analysis of transcriptome data at 24-hr exposure showed that three groups were formed and were separated from each other, indicating that PC1 (32.1%) and PC2 (12.3%) reflected the effects of mixtures and single PAHs, respectively. The profiles of altered gene expressions at 24-hr exposure were similar between phenanthrene- or pyrene-exposed groups; however, the effects of their mixture were completely different from those of individual compounds. Exposures to phenanthrene or pyrene induced the genes related to *immune response* (GO:0006955) and *induction of apoptosis* (GO:0006917) in a dose-dependent manner, which seemed to be an acute response to PAH exposures. Meanwhile, exposures to mixtures of phenantherene and pyrene dramatically suppressed the expression levels of genes associated with *collagen catabolic process* (GO:0030574) and *glycolysis* (GO:0006096), although each individual chemical showed no effect on the expressions of these genes. On the other hand, the PCA result of 96-hr exposed groups did not show the similar trend, and the gene expression patterns exhibited neither dose-response nor PAH mixture-specific effects. The effects of fasting, detected as significant changes in gene expression profiles of control fish among time points. Data analyses using GO terms revealed that 96-hr fasting significantly suppressed *collagen catabolic process* and *glucose homeostasis* (GO:0042593), which were also observed in fish exposed to mixtures of PAHs. The effects of fasting seemed larger than those of PAH exposures at 96-hr, which may be a major factor of interference with the detection of biological responses to PAH exposures. Thus, our analysis can detect mixture toxicities of PAHs, as well as the effects of individual PAHs. However, some effects of PAHs were overlapping with the effects of fasting. Therefore, for precise evaluation of chemical effects by microarray experiment, fasting should be considered as a factor to affect the gene expression profiles in experimental animals.

ET07B-1

Transcriptomics analysis of intersex condition in rainbow darter (Etheostoma caeruleum) exposed to sewage effluents in Grand River, Ontario, Canada

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Rainbow darter (Etheostoma caeruleum) is a small benthic fish found in North America. Rainbow darters are very abundant and spatially distributed throughout the Grand River system (Ontario, Canada) which is a model river for the study the sewage effluent effects on fish populations because of high municipal sewage input. Rainbow darters are sensitive to sewage effluents in this environment, showing the presence of intersex condition in males. The primary objective of this research was to identify molecular pathways that are associated with intersex and to explore the impact of sewage effluent on higher level biological endpoints. In May 2011, we collected female and male rainbow darter at 9 sites in Grand River in a gradient of sewage effluent pollution. There were no significant changes in condition factor, hepatosomatic index (LSI), or gonadosomatic index (GSI), in fish in polluted environments compared with the reference site, but variability was increased downstream. Histology revealed that males had a high incidence of intersex in polluted sites as characterized by the presence of follicles within the testis. Male fish had less 11-Keto-testosterone and testosterone production at the downstream sites. To better understand mechanisms underlying intersex in the rainbow darter, known genes involved in sexual differentiation (sox9b, foxl2 and dmrt1) and reproduction (esr1, erb, ar, vtg, aromatase and cyp11a) were cloned and evaluated with real-time PCR, comparing males, females and intersex fish. In addition, a rainbow darter microarray was developed using 454 pyrosequencing to characterize molecular pathways that are involved in intersex. For example, Sox9b and foxl2 were significantly up-regulated in the intersex condition while dmrt1 keep express on intersex. Vtg expression was significantly increase in intersex in comparison with males from reference and polluted sites. Cluster analysis of microarray data shows individuals that have a more advance intersex condition (i.e. vitellogeninc oocytes) have a very unique gene expression pattern compared to males and females. The microarray analysis between males from reference sites, males from polluted sites and intersex males demonstrate that biological processes that included oogenesis were different in intersex compared to males. Finally, this study identifies novel genes and cell pathways underlying intersex condition in teleosts.

ET07B-2

Sequencing the Chironomus riparius transcriptome to compare the sensitivity of gene expression and life-cycle endpoints to toxicant exposure

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The non-biting midge *Chironomus riparius* (Insecta, Diptera) is routinely used in sediment toxicity tests, where the toxicity of compounds is assessed by observing effects on life-cycle endpoints, such as emergence, survival and growth. This approach has proven to be effective, with currently four OECD guidelines being available for acute and chronic sediment toxicity tests with chironomids. With the rapid developments in sequencing technology, a steady increase is seen in the number of ecotoxicological studies where endpoints on a lower level of biological organization, i.e. gene expression, are used to assess the effects of toxic compounds. The aim of the present study was to develop a *C. riparius* gene expression microarray and compare the sensitivity of gene expression endpoints with life-cycle endpoints in sediment dwelling *C. riparius* larvae. Therefore, we performed 14-day sediment toxicity tests with four compounds, i.e. the essential metal copper, the non-essential metal cadmium, the organometal tributyltin and the polycyclic aromatic compound phenantrene, and measured the effects on growth and survival. EC50 and LC50 values were obtained for all compounds, except for phenanthrene where the surviving larvae were not impaired in their growth, thus no EC50 value could be calculated. To obtain the *C. riparius* transcriptome, a portion of the exposed larvae was pooled with midges of different developmental stages and used to construct a normalized cDNA library for pyrosequencing. This yielded 1549146 sequence reads which were assembled into 23709 isotigs and 135082 singletons. BlastX search and functional annotation with Blast2GO showed that 83% of the isotigs that had a blast result, had the highest homology with dipterans, while 92% matched best with insects in general. Prior to developing the 135K *C. riparius* gene expression microarray, we validated the sequence reads using a comparative genome hybridization and gene expression analysis on a 1000K *C. riparius* microarray. This resulted in a confirmed transcriptome assembly of 20.481 isotigs and 19.021 singletons, and allowed the selection of 135000 reliable probes for the final microarray. This gene expression microarray was used for gene expression studies with the toxicant exposed larvae and will allow us to compare the sensitivity of the molecular and life-cycle toxicity endpoints.

ET07B-3

A novel method for cross-species gene expression analysis - applications for ecotoxicology

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Cross-species comparisons and extrapolations is a cornerstone of ecotoxicological risk assessment. A generalized method that could be applied to the growing number of ecotoxicological relevant transcriptomic studies would therefore be highly valuable in the identification of evolutionary conserved modes of action as well as novel biomarkers of exposure or effect. Indeed, comparing gene expression profiles from different species is a powerful way to identify evolutionary conserved transcriptional responses. However, due to evolutionary events such as gene duplication, there is no one-to-one correspondence between genes in different species and comparison of their gene expression profiles is therefore complex. Here, we have developed a new method for cross-species meta-analysis of transcriptomic data. In contrast to other procedures, our method takes the homology structure between compared species into account and can therefore compare expression data from genes with any number of orthologs and paralogs. A simulation study shows that the proposed method results in a substantial increase in power compared to other previously suggested procedures. As a proof of concept, we analyzed microarray data from heat shock experiments performed in eight species where we identified several well-known evolutionary conserved transcriptional

responses. The method was also applied to five different gene expression studies where fish had been exposed to estrogenic substances. Well-known biomarkers of estrogenic exposure and mechanisms of action were identified together with more novel findings.

ET07B-4

Metabolomics approach to evaluate the effects of polybrominated diphenyl ethers (PBDEs) on the central nervous system and the hypothalamic-pituitary-gonadal axis of marine medaka (Oryzias melastigma)

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Owing to the extensive applications of polybrominated diphenylethers (PBDEs) as flame-retardants, residues of PBDEs can be easily found in the aquatic environment and biota. Resulting from their lipophilic characteristics, PBDEs can be bio-accumulated bio-magnified along the food chain. Although great efforts have been devoted to the study of the environmental and toxicological impacts of PBDEs on the coastal marine ecosystem, our knowledge on their general health effects on marine biota still remains obscure. Because of their ability to pass the blood-brain barrier and to accumulate in the brain, they have adverse effects in the central nervous system in the exposed organisms. Because of the intertwined nonlinear and dynamic interactions among large numbers of cellular components, such as genes, proteins and metabolites, organisms often respond to external stresses and stimuli in complex and unpredictable ways (Nicholson & Lindon, 2008). In order to understand the systemic responses and behaviors of a biological entity, an integrative approach to study and model the pathways and networks involved in the overall functioning of the entity is needed (Henry et al., 2003). Such a system biological approach usually involves the perturbation of the biological system and monitors the resulting impacts at the various "omics"levels, such as its genomics, proteomics and metabolomics.
In this study, we used marine medaka (*Oryzias melastigma*) as a marine vertebrate model organism to study the changes induced by the exposure to BDE-47, a predominant PBDE residue in the marine ecosystem, on the profiles of selected neuro-transmitters and their metabolites. By using a novel LC-ESI-MS/MS metabolomics platform, we were able to evaluate the multi-parametric metabolic responses on central nervous system (CNS) and hypothalamic-pituitary-gonadal (HPG) axis of a living system to PBDEs exposure. Our results indicate that food-borne exposure to BDE-47 is able to induce abnormal expression of a number of neuro-transmitters in the central nervous system of the model organism.

ET07B-5

Metabolomic approach to study the mechanisms of toxicity of a non-ionic surfactant in fish

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Non-ionics surfactants account for 50% of the total surfactant production and they are the second highest synthetic surfactant in terms of worldwide consumption. Between them alcohol polyethoxylates (AEOs) have the highest production volume in EU today, and they have replaced alkylphenol polyethoxylates (APEOs) due to the estrogenic properties of some of their metabolites. After their use, AEOs are released either directly or via wastewater treatment plants into aquatic ecosystems assuming minimal risk for the health of aquatic life. Although AEOs don´t present estrogenic properties as APEOs, there is little information on the effect of exposure to sub-lethal concentrations and the possible disruption on biochemical pathways of organisms that could adversely affect their health. In order to investigate this topic, a metabolomic profiling approach was used to characterise changes in tissues and biofluid of *Solea senegalensis* exposed to the non-ionic surfactant hexaethylene glycol monododecylether (C12EO6). Fish were exposed to two different concentrations of the chemical in a flow-through system for 120 h, followed by a 72 h of depuration period. Blood, liver and gills were extracted with methanol and the metabolites present in the extracts were profiles using LC-TOF-MS. Analysis of extracts revealed a high number of metabolites produced from the phase I and phase II biotransformation of the parent compound by the organism. Metabolomics analyses also revealed that the biochemistry of internal molecules was affected by the surfactant exposure. Increased concentrations of cortolone-3-glucuronide, and bile acids were measured in plasma and identified as potential endogenous markers of surfactant exposure. The concentrations of taurocholic acid, hydroxytaurocholic acid, cyprinol sulphate and scymnol sulphate increased in plasma by up to 10^8 times higher after exposure indicating that hepatoxicity may have resulted from cholestasis and impeded flow of bile from liver to the intestine. Plasma levels of L-palmitoylcarnitine decreased by up to 10^{-3} fold as a result of surfactant exposure indicating affects on the metabolism of fatty acids. After the depuration the levels of L-palmitoylcarnitine were re-established indicating that the normal metabolism of fatty acids was recovered but the concentrations of bile acids kept increasing which showed that the damage caused to the organism was still present.

ET07B-6

Metabolism of and adaptation to arsenic in the earthworm, Lumbricus rubellus

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As contamination in the terrestrial environment can have deleterious effects upon the flora and fauna exposed. The persistent nature of the metalliod provides a strong selective pressure that may lead to the occurrence of adapted populations. While certain organisms demonstrate tolerance, others are able to acquire genetically based resistance, whereby survival is ensured following exposure to concentrations that are normally lethal. Such resistance may come at a price, with reduction in genetic variability following speciation events potentially increasing the sensitivity of adapted species to future environmental stress, while diverting resources limiting overall fitness. It is therefore important to determine the sensitivity of populations and potential ecological implications of resistance. Our investigations compare exposure sensitivity to metals among naïve and tolerant populations of the earthworm Lumbricus rubellus and evaluate the capacity for gene flow within contaminated environments via integration with phylogenetic data. Furthermore, we are employing a systems biology analysis (metabolomics and transcriptomics) to clearly resolve As metabolism within naive L. rubellus that will supplement investigations for the demonstration of genetic mechanisms adopted by adapted individuals using Restriction Associated DNA sequencing (RADseq).

ET08 - Extrapolation within wildlife toxicology

ET08-1

Increasing the reliability of the chemical effect assessment for wildlife with interspecies correlation estimations

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The median hazardous dose of a chemical for warm-blooded species (HD50) is one of the key characteristics in the relative ranking of toxic chemicals. Generally, experimental information is available for a limited number of warm-blooded species only, which causes statistical uncertainty. Furthermore, when small datasets contain an unrepresentative sample of species, they can cause systematic uncertainty in chemicals' median lethal doses. The number of species can be enlarged with interspecies correlation estimation (ICE) models, but these are uncertain themselves. The goal of this study is to quantify the possible gain in reliability of the HD50 values for warm-blooded wildlife species after enlargement of the sample size with ICE predictions. For 1137 chemicals, we compared potential systematic uncertainty and statistical uncertainty between HD50s based on experimental data (HD50Ex) and datasets combining experimental data and ICE predictions (HD50Co). HD50Ex values ranged between 0.10 and 9,500 mg-kgwwt-1, and HD50Co between 1.1 and 6,100 mg-kgwwt-1. For over 97 percent of the chemicals, HD50Ex values exceeded HD50Co values, with a systematic uncertainty (i.e. the ratio of HD50Ex/HD50Co) of typically 3.5. This finding suggests that the limited availability of mammalian, experimental toxicity data results in a systematic underestimation of the wildlife toxicity of a chemical. Statistical uncertainty factors (i.e. the ratio of the 95th/5th percentile) ranged between 4.8-100 and 1.1-102 for the combined dataset, but between 1.0-100 and 2.5-1022 for the experimental dataset. For thirty percent of the chemicals, enhancement of the toxicity with ICE predictions gave a reduction in statistical uncertainty of up to twenty orders of magnitude, for the remaining seventy percent a combined dataset resulted in statistical uncertainty of maximally two orders of magnitude. The supplementation of experimental toxicity data with ICE predictions makes it possible to eliminate large outliers of statistical uncertainty. We conclude that both systematic uncertainty in chemicals' HD50 values and statistical uncertainty can be reduced by supplementation of experimental data with ICE model predictions, particularly in cases of limited toxicity data ($n \leq 4$) for mammals only.

ET08-2

Contribution of wintering area to levels of organochlorines and polybrominated diphenyls in plasma of an avian top predator in the North Atlantic

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Top marine predators, including seabirds, are often sampled to monitor levels of persistent organic pollutants (POPs) in the marine environment. Despite the restrictions on use and production of many POPs and declines in some legacy POPs in the environment, concern remains about levels of these chemicals present in marine biota due to their potential toxicity. Many species of seabird are migratory, but there is a lack of studies investigating the possible contribution of time spent in their wintering area to their POP burden. Advances in tracking technology now mean it is possible to link the POP levels in an individual bird to their wintering area, recorded using geolocators. The great skua (Stercorarius skua) was chosen as the study species as it is a top predator and has breeding populations that contain individuals which winter in geographically discrete locations over a large geographic area. Geolocators were deployed in three breeding colonies in 2008, in Scotland (Foula; n=16), in Southeast Iceland (Öræfi; n=40) and in

Svalbard (Bjørnøya; n=24) and 20 were recovered in 2009. Carbon isotope signatures differed between birds wintering in different areas. The δ13C signature of the eighth primary feather sampled from each logger bird from a known wintering area was used to classify the wintering area of a wider population of breeding individuals from the three colonies. We assessed the influence of wintering area on POP levels in plasma of birds sampled during the breeding season. All Scottish birds wintered in the eastern Atlantic. Icelandic great skuas wintering in the western Atlantic had significantly higher levels of organochlorines (OCs) and Polybrominated diphenyls (PBDEs) than those wintering in the eastern Atlantic. In the Bjørnøya population there was no difference in OC or PBDE levels between birds wintering in the east and west Atlantic. This may due to differences in diet between the Icelandic and Bjørnøya breeding populations, the latter birds feed at a higher trophic level during the breeding season than the other two colonies and having significantly higher POP levels than Icelandic and Scottish great skuas. Although breeding colony significantly affects POP levels, wintering area also contributes to explaining POP levels in birds and should not be neglected.

ET08-3

Responses of wild small mammals to arsenic polluted soils on a partially remediated mining site in Southern France

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Arsenic (As) is a chemical element naturally occurring in soils but industrial and mining activities have long been releasing high As levels into the environment. These elevated concentrations might affect directly exposed organisms and also organisms that feed on them along the food chain. Despite this, little is known about As behaviour in the environment. Chemical analyses of soils provide information on contaminant concentration but not on bioavailability, transfer, and toxicity to wildlife, all being critical points for ecological risk assessment (ERA). In As case, transfers highly depend on environmental matrices, thus a local estimation of As bioaccumulation is needed for ERA. On a partially remediated site, we measured (i) As concentrations in soils to estimate the efficiency of remediation works and (ii) As bioaccumulation in small mammals to improve knowledge of As transfers within food web and of associated risks.

The study site is a partially remediated former gold mine in southern France (Salsigne, Aude, France). In 45 soil samples, As concentrations and soil characteristics were measured. Small mammals were captured with baited snap-traps, and animals were identified at species level, sexed, weighed and measured, and age of rodents was estimated from the eyes lens weight. As concentrations in organs were quantified by graphite furnace atomic absorption spectrophotometry.

Exploitation of the rich ores in the study area led to high As soil concentrations (median NZ4 = 17,900 ppm). The two kinds of remediation works (by clean soil addition or by As immobilisation and phytoremediation) resulted in different soil As levels between zones: some were similar to the control site and the other were similar to non remediated areas (with the exception of an extremely contaminated zone, NZ4). Small mammals living there showed As accumulation in liver, kidneys and lungs among the highest recorded in the literature (e.g. As median in kidneys wood mouse on contaminated area = 5,3 ppm with a maximum of 51 ppm). Discrepancies in As concentrations spatial patterns between soils and organs may be a reflect of the foraging activity of animals. The analysis of small mammal's diet, currently in progress, should help to test this hypothesis. Finally, the high internal As concentration call for an analysis of the effects of As on small mammals, both at the individual and population levels, which are also in progress and will be soon available.

ET08-4

Assessing the exposure and effects of persistent contaminants in river otters (Lontra candensis) in Victoria Harbour, Vancouver Island, British Columbia

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Monitoring contamination in wild otter populations has proven challenging due to their elusive nature and the invasive sampling techniques traditionally involved (e.g. trapping and tissue sampling).

Elliott et al. (2008) recently reported that scats collected from river otter latrines in Victoria Harbour, BC, Canada contained levels of PCBs that exceeded 9 mg/kg lipid, a published criteria for reproductive impairment in mustelids. Guertin et al. (2010) applied an individual based approach by combining fecal DNA genotyping with contaminant and diet analysis of individual river otter scat samples from the Victoria Harbour population. This approach revealed variation in individual contaminant exposure across the landscape along a gradient from industrialized to natural near shore environments. Genetic data also indicated small scale population structuring, suggesting that only certain otters (one subpopulation) were being exposed to contaminants.

This study combined live animal sampling and radio-telemetry with non-invasive scat sampling. The objective was to evaluate the reliability of individual, population and contaminant data derived from river otter scat. This approach was also well suited for defining individual home ranges and characterizing contaminant exposure in this population. Individual river otters were radio-tracked to investigate home range, distribution and movement patterns. Telemetry data reveals that individual river otters inhabit home ranges that span approximately 5kms of coastline. Movement patterns indicate that there is limited if any mixing between the proposed subpopulations and that certain otters inhabit the contaminated areas year round. Understanding the movement patterns and ranges of these individuals will help to further investigate bioaccumulation of contaminants from prey to predator as well as any physiological effects from this exposure. The data derived from feces will be compared to data derived from river otter blood and tissue to investigate bioaccumulation of contaminants from prey to predator as well as any physiological effects from this exposure.

ET08-5

Anticoagulant rodenticides in New Zealand birds - so what?

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The focus for monitoring residual concentrations of anticoagulant rodenticides in wildlife has largely been on birds of prey and mammalian predators. Here we report the results of such monitoring undertaken in New Zealand (NZ) during 2009-2010, predominately of harrier hawks (Circus approximans) but including other bird species. Concentrations of multiple coumarin anticoagulants (brodifacoum, bromadiolone, flocoumafen coumatetralyl and warfarin) were measured in liver tissue using a HPLC method. Residues of at least one anticoagulant were detected in 22 out of 27 harrier hawks. Three hawks had one anticoagulant only, and about half (13 of 27) had two anticoagulants present, most commonly brodifacoum and flocoumafen. Three anticoagulants were present in four of the 27 hawks, and four anticoagulants present in another two hawks. Overall, brodifacoum (n=18) and flocoumafen (n=17) were the most frequently detected.

These data add to the growing international literature describing the prevalence of anticoagulant residues in non-target wildlife, and also highlight the recurrent question of 'so what?' We propose that a laboratory trial using a model bird species and repeated dietary exposure to anticoagulants would be an appropriate first tier step in addressing this question. The aim would be to characterise levels of harm, describe how liver concentrations correspond to risk of mortality and determine the repeated exposure patterns that could ultimately produce a mortality 'tipping point' for individuals.

ET08-6

Incidence of poisoning by anticoagulant rodenticides in non-target wildlife and domestic animals in Spain

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Anticoagulant rodenticides (ARs) levels were studied in liver of 401 found dead wild and domestic animals in Spain with evidences of AR poisoning, including 2 species of reptiles (n=2), 42 species of birds (n=271) and 18 species of mammals (n=128). Baits (n=32) were also analyzed to detect the potential use of ARs in their intentional preparation to kill predators. AR residues were detected in 38.2% of the studied animals and 34.9% may have died by AR poisoning according to the clinical information, necropsy findings, residue levels and results of other toxicological analysis. Animals considered with sublethal AR exposure had total AR residues levels (geometric mean with 95% CI) in liver of 0.005 (0.003-0.007) µg/g and animals diagnosed as dead by AR poisoning had 0.706 (0.473-1.054) µg/g. ARs were detected in 19% of baits illegally prepared to kill predators. In terms of the total incidents studied in our laboratory between 2005 and 2010 (n=1,792 animals), confirmed poisonings represented 40.9% of the cases, and 20.7% of these were due to ARs (8.5% of the total sample). Nocturnal raptors (62%) and carnivore mammals (38%) were amongst the groups with higher prevalences of secondary AR exposure, especially to second generation ARs (SGARs). On the other hand, granivorous birds showed the highest prevalences of primary AR exposure (51%), especially to chlorophacinone in a region treated against a vole population peak in 2007. The presence of haemorrhages was significantly associated with AR levels in liver, but some animals (7.2%) with elevated residue levels (>0.2 µg/g) showed no evidence of macroscopic bleeding. Previous toxicovigilance studies have found a prevalence of 26.9% of AR poisoning within confirmed cases of poisoning in non-target animals (mainly pets) in Spain. Further current monitoring studies of AR in particular species of wildlife in Spain are also discussed. The use of accumulative SGARs and the application of baits on surface (i.e. treated grain by spreader machines) should be discontinued in future EU regulations on the use of rodenticides to prevent the poisoning of non-target wildlife species.

ET09 - Global climate change: implications for environmental toxicology and chemistry

ET09-1

Global climate change and influence on chemical fate and bioavailability

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A SETAC Pellston workshop, held in July, 2011, brought together an international group of experts in environmental chemistry, toxicology, ecology, human health and risk assessment, to advance the level of scientific understanding of how global climate change may affect the environmental fate, bioavailability and toxicity of chemicals in the environment. Six workgroups addressed a range of areas that may be affected by climate change. The emphasis of workgroup 1 was aimed at assessing the influence of climate change on exposure, fate, transport, and deposition of chemicals in the environment, and how potential changes may impact environmental risk and hazard assessments.
A key challenge identified relates to the complexity of interconnected environmental processes that may result in enhanced or reduced exposure of chemical contaminants. The complexity of the interactions can lead to non-intuitive results, making projections of how chemical fate and bioavailability might change in a world impacted by GCC difficult to assess. Nevertheless, efforts are made to demonstrate how models can quantify the physical-chemical properties of substances that may be more sensitive to changes in climate than others. Additionally, we attempt to demonstrate which global regions enhanced chemical exposure is more likely to occur. Utilizing an appreciation for the uncertainty and sensitivity of model parameters, as defined for both environmental fate models and climate change models, we attempt to demonstrate how model output can complement the collection of empirical data in a manner that can better inform our ability to assess potential relationships between chemical fate and bioavailability and changes in climate that can be attributed to climate change.

ET09-2
A pathway-based approach to predicting interactions between chemical and non-chemical stressors: applications to global climate change
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Incorporation of global climate change (GCC) effects into assessments of chemical risk requires an understanding of effects on mechanisms of chemical toxicity. These effects could include changes in toxicokinetics (chemical absorption, distribution, metabolism and excretion), as well as alterations in toxicodynamic interactions between chemicals and target molecules. GCC also can modify an organism's baseline physiological processes for coping with the external environment (e.g., water balance, thermoregulation, immune-endocrine-neurological systems). In organisms living in GCC modified climates, increased investment in adaptation could lead to heightened susceptibility to chemicals, disease and other stressors. Basically, GCC can cause organisms to be more sensitive to chemical stressors while, alternatively, chemical exposure can make organisms more sensitive to non-chemical (GCC) stressors. Implications of GCC interactions with chemical mechanisms of toxicity are applicable to both ecological and human health effects assessments. To better address the complexities of interactions between chemical and non-chemical stressors, we employed adverse outcome pathways (AOPs), constructs that depict linkages between molecular initiating events and subsequent responses occurring across biological levels of organization, culminating in impacts in individuals or populations that can be used for assessing risk. Through a series of examples and case studies, we demonstrate how chemical- and climate-specific interactions could lead to adverse outcomes. Scenarios are prospective, which project outcomes based on known or anticipated chemical/GCC interactions, as well as retrospective, where mechanisms are proposed for known or demonstrated chemical-climate interactions. Understanding GCC interactions along AOPs provides opportunities for extrapolation across species and biological levels of organization, and between different exposure scenarios, facilitating development of hypotheses and focal areas for further research, and improved inputs for risk assessments.

ET09-3
Combined impacts of global climate change and toxicants on populations and communities
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SETAC convened a workshop in July 2011, organized into 6 workgroups, to address a variety of issues associated with the potential impacts of global climate change (GCC) on chemical fate, exposure, toxicity and risk assessment. Climatic changes such as higher temperature and CO2 levels have documented negative impacts on many species, e.g. amphibians and coral reefs. GCC-related stress and toxicant exposure can therefore be regarded as multiple stressors, where the combined effects can result in synergistic or antagonistic interactions. Moreover, GCC can also directly and indirectly impact on population and community processes, e.g. disrupt the timing of predator-prey interactions. A great challenge for ecological risk assessment is to predict the how toxicants effects at the individual level (e.g. reduced survival) will be transferred to the population level (e.g. population growth rate) or community level (e.g. species richness). Given the large complexity of direct and indirect GCC impacts on the environment, it is not possible to give general predictions for combined climatic and toxicant impacts will propagate from the individual to higher ecological levels. Our approach in this paper, therefore, is to describe relevant ecological mechanisms that will influence the responses of ecosystems to toxicant stress under climate change. In particular we will focus on these topics: (1) Resistance, resilience and recovery from disturbances; (2) Acquired tolerance to stressors and associated costs; (3) Species traits and vulnerability in a landscape context. Within this framework, we use case studies from various aquatic ecosystems to illustrate the complexity of joint effects of GCC and toxicants on populations and communities.

ET09-4
How will global climate change affect human health risk assessment?
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Global climate change is predicted to alter long-term weather characteristics in different regions. These changes, including increased temperature, greater precipitation extremes, and loss of glacial and polar ice, have implications for human exposure to chemical contaminants. Climate change may also directly and indirectly affect the vulnerability of humans to chemical exposures. Changes in human exposure may arise from altered use, inputs, fate and transport of chemicals due to climatic and other drivers. Human vulnerability may be affected directly by heat and other weather-related stressors, or indirectly through altered co-exposures or disease patterns.
To further explore the implications of climate change for the assessment and management of chemical risks, the authors examine four specific risk contexts: natural toxins, pesticides, air pollutants, and legacy chemicals (e.g., mercury, POPs). For the specific types of decisions to be made in each of these contexts, we examine how assessments and management decisions may be affected by climate change, and how significant the impacts of climate change may be.
Climate change is likely to both increase and decrease human exposures, depending on the specific contaminant and specific region or other exposure context. There is limited evidence that climate change will increase the sensitivity of humans to chemical exposures. But small changes in exposure variability or human vulnerability can translate into significant changes in population risk profiles.
To assess and manage chemical risks effectively, exposure data sources will need to be regularly updated and defaults and assumptions used in exposure assessment evaluated in a context of changing climate. Monitoring and sampling should be done with frequency sufficient to capture variability, which is likely to increase in many places. There are many research gaps in interactions between climate and weather parameters and human responses to chemical exposures. These factors will all exacerbate gaps in chemical protection between developed and developing countries.

ET09-5
Ecological risk assessment in the context of global climate change
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This paper originates from the SETAC Pellston Workshop entitled "The Influence of Global Climate Change (GCC) on the Scientific Foundations and Applications of Environmental Toxicology and Chemistry"and specifically, from the workgroup charged with determining how ecological risk assessment (ERA) will encompass the

challenges presented by GCC. Although the basic ERA structure remains useful, the process needs to broaden beyond contaminant issues per se to include interactions with changing habitats that alter biologic communities and recognize that landscape ecology needs to be embraced to optimize effective environmental management decisions in the context of GCC. We propose seven principles for conducting ERAs under GCC: 1) evaluate a priori whether climate-related factors are likely to impact to a given ERA process and management decisions; 2) develop and express assessment endpoints as ecosystem services; 3) recognize that responses, (changes in ecosystem services), can be positive or negative; 4) recognize that the risk assessment process requires a multiple stressor approach with non-linear interactions; 5) develop conceptual cause-effect diagrams that consider context-dependent management decisions, at the appropriate spatial and temporal scales while ranking direct and indirect effects; 6) determine the major drivers of uncertainty by estimating and bounding stochastic uncertainty spatially, and across time; and, 7) plan for adaptive management to account for changing environmental conditions and consequent changes to ecosystem services. Given the complexities and uncertainties associated with GCC, good communication is essential for making risk-related information understandable and useful for managers and stakeholders.

ET09-6

Environmental contaminants and global climate change: implications for environmental damage assessment and restoration/rehabilitation

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A SETAC international workshop, held in July, 2011, brought together an international group of experts in environmental chemistry, toxicology, ecology, human health and risk assessment and natural resource damage assessment, to advance the level of scientific understanding of how global climate change (GCC) may affect the environmental fate, bioavailability and toxicity of chemicals in the environment. Six workgroups addressed a range of areas that may be affected by GCC. The emphasis of the restoration workgroup was environmental damage assessment and restoration/rehabilitation in relation to GCC, specifically addressing: (i) legislation relating to contaminant management, damage assessment and restoration/rehabilitation requirements; (ii) experience with damage assessment and restoration implementation; and (iii) potential direct and indirect influences of GCC on damage assessment and restoration/rehabilitation processes. An example is provided in the U.S., where legislated requirements for damage assessment and restoration incorporate chemical exposure and ecosystem effects from site contaminants to assess the injury to resources and estimate damages paid for restoration of those resources. GCC will impact the processes of assessing injury and rehabilitating/restoring and conserving resources by affecting the magnitude of impact of contaminants on natural resources and altering potential restoration/rehabilitation efforts. Baseline/reference conditions for estimating resource injury and restoration/rehabilitation also may shift significantly and exhibit greater variability due to GCC, representing a significant challenge to practitioners. This presentation will discuss how such future environmental damage assessments and restorations might be influenced by GCC and provide recommendations of research needs in the field.

ET10 - Long-term ecotoxicological impact: trans-generational effects and evolutionary responses to pollutants

ET10A-1

How evolutionary concepts may enhance ecotoxicology: tracing the genetic background of differential cadmium sensitivities in invertebrates

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Species sensitivity towards pollutants and risk assessment parameters are often regarded as random variables in a statistical approach. Beyond statistics, however, toxicant sensitivity of every species assumes a biological significance, especially if we consider that sensitivity traits have developed in lineages of species with common evolutionary roots. The significance of such a perception will be stressed by reviewing and reporting examples of work from our own lab.
In the first case we explored the possibility that populations of Tubifex tubifex from different European rivers (Danube, Oder and Dnjepr) may exhibit differential sensitivities towards Cd. This hypothesis was based on the fact that the populations had developed in river systems that are geographically separated and may have experienced, in addition, different contamination histories during the past decades. Populations were screened for Cd toxicity and a mitochondrial gene marker (16S rDNA). All natural populations contained, to different degrees, distinct genetic lineages or even cryptic species of Tubifex differing significantly with respect to their Cd sensitivity. This suggests to consider, whenever possible, potential genetic differentiation in populations or species when performing ecotoxicity experiments, or when evaluating ecotoxicity data for purposes of risk assessment. We are currently planning to investigate the physiological basis behind these intra-specific sensitivity traits in tubificid worms.
The second example is focused on the evolution of Cd-specific MT isoforms in terrestrial snails. In this case, evolution has led to functional diversification of MT isoforms through shaping of their metal-specific features in a large monophyletic group (the pulmonate snails). As a result of this evolutionary process, terrestrial gastropods possess a Cd-specific MT isoform that must be regarded as a major trait conferring enhanced Cd tolerance to these animals. Interestingly, however, emerging differences evolved in both, regulatory and coding regions of pulmonate CdMT genes give rise to differential transcriptional regulation and expression of deviating gene products in a population- and species-specific manner. This may have implications on Cd tolerance of the affected individuals.
It is concluded that both, differences and similarities in sensitivity traits of related invertebrate species can only be understood if we also consider the underlying evolutionary processes and mechanisms.

ET10A-2

The potential for adaptation in a natural Daphnia magna population: narrow-sense heritability of net reproductive rate under Cd stress at two temperatures

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The existence of genetic variability is a key element of the adaptive potential of a natural population to stress. In this study we estimated the additive and non-additive components of the genetic variability of net reproductive rate (R0) in a natural Daphnia magna population exposed to Cd stress at two different temperatures. To this end, life-table experiments were conducted with 20 parental and 39 offspring clonal lineages following a 2[GREEKX]2 design with Cd concentration (control vs. 3.7 µg Cd/L) and temperature (20°C vs. 24°C) as the factors. Offspring lineages were obtained through inter-clonal crossing of the different parental lineages. The population mean, additive and non-additive genetic components of variation in each treatment were estimated by fitting an Animal Model to the observed R0 values using restricted maximum likelihood estimation (REML). From those estimates narrow-sense heritabilities (h2) of R0 were calculated. Significant values of h2 (=0.23) were only found in the 24°C + 3.7 µg Cd/L treatment, suggesting that the ability to produce more offspring under this stressful condition may be inherited across sexual generations. In the three other treatments these values were all low (h2 range: 0.04-0.06) and not significantly higher than 0. Collectively our data indicate that both the asexual and sexual reproduction phases in cyclic parthenogenetic D. magna populations may play a role in the long-term adaptive potential of Daphnia populations to chemical stress (with Cd as the current example) and that environmental variables other that the chemical itself may influence the adaptive potential to that chemical (with temperature as the current example).

ET10A-3

Do pesticides influence evolutionary processes in natural populations of non-target species? A study in the freshwater snail Lymnaea stagnalis

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Pollutants present in the environment may affect the evolution of natural populations in several ways. Fitness reduction can result from direct effects on the germ-line (mutagenic compounds), or it may be the consequence of a negative impact on genetic diversity, through directional selection (when the population becomes locally adapted) or through amplification of random genetic drift (when local demography and dispersal patterns are impaired). Pollutants may thus affect the evolution of genes under selection as well as neutral regions of the genome. The issue of evolutionary impact is therefore conceptually important for ecotoxicologists and also for ecological risk assessment and management.
With respect to human induced pollutions, some conditions are expected to increase the risk of genetic change in natural populations. Among such conditions, freshwater lentic habitats located within agricultural landscapes are likely to be exposed to recurrent contamination by pesticides, through various modes of transfer from the treated parcels. Non-target species occupying these habitats are thus exposed to a high risk of evolutionary impact, especially when they have low dispersal ability (e.g., when the whole life cycle is aquatic) or opportunity (weak connectivity among occupied sites, e.g., marshes or ditches).
The present study investigated the effect of historical exposure to pesticides on population genetics patterns, using the freshwater gastropod Lymnaea stagnalis as model species. A set of 14 populations was sampled from two contrasted types of landscape, expected to differ in terms of pesticide pressure (sites within zones of intensive agricultural activity vs sites distant from such zones). Patterns of population genetic differentiation were compared between neutral genetic markers (microsatellites) and quantitative traits related to fitness (life history traits) for the two types of populations. Additionally, the project includes a quantitative analysis of population divergence for gene expression at the transcriptome level (RNAseq, qPCR). The aim of the study was to test the hypothesis that chronic exposure to pesticide cocktails had a selective effect on the studied populations. The applicability of the presented approach to ecological risk assessment will be discussed.

ET10A-4

Rapid evolution in a Caenorhabditis elegans (nematodes) population: evaluation of resistance costs

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Anthropogenic disturbances affect more and more the evolution of contemporary populations. Pollution, one of the anthropogenic stress, amplifies the intensity of selection pressures which occur in natural populations. Consequently, micro-evolutionary changes may occur very quickly in response to these new forces. Environment can usually undergo simultaneous changes in various conditions, and this will likely increase with the increase in human activities. These changes may accentuate stress, affecting the life, the growth and the reproduction of individuals, with strong demographic and longer-term phenotypic and genetic consequences.
We started knowing the evolutionary response of a population to changes in one environmental parameter. However, much less is known on the evolutionary reactions of a population to more realistic, multi-variate changes in environmental conditions. We therefore investigated firstly the evolutionary responses of *Caenorhabditis elegans* population exposed to uranium (U) or salt (NaCl) stress for 22 generations. We confirmed the adaptation to stressed population through fitness increasing for both polluted selection regime. In parallel, common garden experiments in control confirmed resistance costs associated to genetic divergence. Indeed individual fitness was lower from NaCl and U-populations. Moreover we conducted a reciprocal transplant experiment with U and NaCl from the generation 18. We found resistance costs of the primary selection regime in other stress environment. NaCl-populations reduced their brood size in U compared to control-populations, but it is not true for U-populations in NaCl. Consequently, There can be an addition of new stress that populations suffered and the action of past selection regime on the reduction of genetic diversity. However, we still need to understand the selection mechanisms.
These results confirm that a population can be affected by pollutants even after contamination will, for instance, be diluted in environment. They also highlight the importance of taking into account the adaptive effects of pollution on population in ecological risk assessment.

ET10A-5

Parental exposure to genotoxicant leads to reproduction impairment in the three-spined stickleback

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The aquatic environment is considered as the ultimate receptacle for anthropogenic compounds that frequently exhibit a genotoxic potential towards organisms. The reproductive process could influence the recruitment rate and hence the population dynamics. The link between the loss of DNA integrity in spermatozoa following paternal exposure to genotoxicant and reproduction impairment has been recently investigated in two fish species. These results have demonstrated a decrease in the progeny survival when sperm DNA was damaged and so, possible long term effects of environmental genotoxicant in aquatic systems. Such results needed to be confirmed through studies carried out in other fish species and the contribution of oocytes to further progeny defects had to be highlighted, as recently done in aquatic invertebrates. The aim of the present study was to investigate the link between DNA damage and reproductive impairment after in vivo exposure of stickleback to the genotoxic compound methyl methane sulfonate (MMS) during the breeding season, paying special attention to the contribution of genetic load brought by each sex to the observed progeny defects.
Adult fish were acclimated and were then exposed or not through water to different MMS concentrations (0 / 0.05 µM / 0.5 µM / 5 µM). In vitro fertilization were realized with mature fish and DNA damage in spermatozoa and erythrocytes (as a biomarker of exposure for both sex) were assessed by the comet assay. The morphological abnormalities of larvae due to parental MMS exposure were studied and the progeny survival at embryogenesis and larval development key stages was evaluated.
Parental exposure of three-spined stickleback to the genotoxicant MMS induced a significant decrease in early life stage survival which was less important when only females were exposed. When males or both genders were exposed, DNA damage level measured in spermatozoa ($p<0.01$) and exposure duration ($p<0.01$) were shown to be significant factors associated with progeny mortality. Whatever the gender, parental exposure to MMS resulted in a 60 fold increase in progeny abnormality frequency.
This study underlines that male genotoxic message transmitted to progeny could predominate on the female one. Hence, this work confirms that spermatozoa are susceptible to accumulate DNA damage under chronic and low-dose exposure to genotoxic compounds, leading to drastic offspring defects in stickelback, as recently shown in other aquatic species.

ET10A-6

Genotoxicity, fertility reduction and regeneration in a multigeneration study using zebrafish after chronic exposure to an alkylating agent

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There is still a significant lack of understanding the mechanisms linking genotoxic effects of anthropogenic pollutants to the decline of fish populations in aquatic ecosystems. Anthropogenic chemical pollution is probably a key aspect of the problem. For a better understanding, investigations into the relationship between genotoxicity and detrimental effects on reproduction in fish are required. For this end, there is a need to further focus on DNA fragmentation and micronucleus formation in somatic and - above all - reproductive tissues as well as on development and spawning capacities of exposed fish and their offspring. In the present study, zebrafish were exposed in vivo to 2 and 0.2 mg/L of the alkylating model genotoxin methyl methanesulfonate (MMS) from fertilisation to the age of one year in a continuous flow-through system. F0 fish were mated over six months to check for first reproduction, egg number and fertilisation rate. Embryonic and larval development of the F1 generation was analyzed daily. In addition, primary cells were isolated from the F0 gonads, liver and gills for analysis of DNA fragmentation in the alkaline comet assay. Micronucleus frequency was measured in histological sections of the same organs. Analogously to the F0 generation, F1 fish were mated. In the F1 and F2 generations, larval development and survival were recorded. In contrast to the F0 generation, F1 fish grew up without exposure in order to allow for regeneration. The time point of the first spawning in MMS exposed F0 fish was delayed. Over subsequent months, the egg production in exposed fish increased, but remained lower than in controls. Weight and length of MMS-exposed fish as well as larval survival in their offspring were reduced. In the comet assay, significant genotoxic effects were found in liver cells from both sexes, female gills and male gonadal cells. Exposure of zebrafish (F0) to MMS thus leads to DNA fragmentation in several organs, delay of spawning, reduction of egg production, as well as to reduced larval survival of the F1 generation. This suggests that anthropogenic genotoxic compounds may play a considerable role in the decline of field fish populations. Currently, micronucleus formation and carcinogenesis in chronically exposed fish as well as regeneration of reproduction parameters in F1 fish are under investigation.

ET10B-1

Patterns of mitochondrial and nuclear polymorphism and DNA -methylation in an arsenic tolerant earthworm population

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Evidence is emerging that earthworms can develop tolerance to trace element enriched soils, thereby allowing populations to exist at polluted sites. There have so far been few studies that have sought to establish the basis of such tolerance earthworms, and in particular whether it is adaptive or evolutionarily based. Here we report a analysis which combines mitochondrial (cytochrome oxidase II) and nuclear (amplified fragment length polymorphism) genotyping and analysis of genome methylation analysis to investigate whether previous observation of arsenic tolerance in the earthworm L. rubellus can be ascribe an underlying mechanism. Both mitochondrial and nuclear genotyping indicate that L. rubellus is a complex of two major cryptic lineages. Mitochondrial genotyping failed to indicate population selection, but AFLP analysis, especially when analysed by lineage, highlighted genome differences that were associated with site physiochemical properties (including metal/metalloid concentrations). DNA methylation was strongly influence by pollutant exposure, clearly separation mine site and reference site populations. Patterns of DNA methylation showed greatest divergence between the control and intermediately polluted sites, suggesting that epigenetic change may be a prevalent response at intermediate pollution levels. Taken all together these results suggests that earthworm adopt a multi-layer strategy to cope with adverse soil conditions, employing epigenetic mechanism to potentially change gene expression possibly as an initial adaptive mechanism and undergoing micro-evolution that can be linked to the development of tolerance traits.

ET10B-2

Genomic mechanisms of co-tolerance of cadmium-adapted Daphnia pulex populations to cyanobacterial stress

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Natural populations are characterized by genetic variability which may allow populations to adapt to a stressor, which is manifested by an increased tolerance to that stressor. As a consequence of this genetic adaptation, populations may become more (co-tolerant) or less tolerant (cost-of-tolerance) to other stressors. In aquatic ecosystems, stressors rarely occur isolated and the number of stressors are predicted to only increase with global climate change (e.g. cyanobacteria). This study aimed to unravel the genomic basis of the co-tolerance of Cd-adapted *Daphnia pulex* to cyanobacterial stress.
Gene-expression profiles of a Cd adapted and a non-Cd adapted clone in response to *Microcystis aeruginosa* (MC, a cyanobacterium) were characterized using a micro-array platform. Molecular pathway analysis was performed on significantly differentially expressed genes based on KEGG Orthology classification. Genes in pathways and sets of

multi-copy genes that were regulated by MC in different ways in both clones were validated through real-time qPCR in an extended set of 14 clones. Additionally, expression of genes found to underlie the differences in Cd tolerance between Cd adapted and non-Cd-adapted clones was studied. These genes may also be involved in the among-clone differences in MC tolerance due to co-tolerance with Cd.
Molecular pathways and sets of multi-copy genes that were regulated by MC in different ways in both clones were the oxidative phosphorylation and the ribosomes pathways, gluthathione transferases, trypsins, neurexins and Apoptosis Inducing Factors (AIF). qPCR expression analysis resulted in three genes for which expression correlated with MC tolerance. Such correlation of a trypsin and an AIF validates the involvement of these genes or the pathways which they belong to in the tolerance to MC. Expression of Keap1, an inhibitor of oxidative stress response tended to be more downregulated in more MC sensitive (thus Cd-non adapted) clones.
This study showed possible genomic mechanisms of co-tolerance of Cd-adapted *D. pulex* populations to cyanobacterial stress. As micro-evolutionary changes in populations and their consequences (such as co-tolerance) for the toxicity of unrelated stressors gain more and more interest in risk assessment, it is benificial to understand the mechanisms that drive these consequences. E.g., if we understand mechanisms of co-tolerance we can perhaps ultimately predict which combinations of stressors may show co-tolerance.

ET10B-3
Multi-generational exposure of Folsomia candida to Cd: survival, reproduction and metallothionein gene effects
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Few studies are currently available on multi-generational effects, particularly with soil invertebrates. In the present study, the effects of cadmium multigenerational exposure were studied in Folsomia candida. Organisms were exposed consecutively to cadmium [EC10 and EC50] along 23 generations of 28 days. Assessed endpoints were survival, reproduction and size. Additionally, the expression level of the metallothionein coding gene has been measured by quantitative real time PCR. Continuous exposure to a concentration of 32 mg Cd/kg (EC10) caused an improvement in performance until F6, after which it started to decline and failed at the 12th generation. Continuous exposure to a concentration of 60mg/kg (EC50) caused an improvement in performance (total number of juveniles) until F6, after which it started to decline but continued at lower level. Population changes could be related to the Mt induction, as continuous exposure to Cd triggered Mt induction which corresponded to reproduction in higher numbers. Interestingly, the higher tolerance observed in the population exposed to the EC50 in comparison to the one exposed to the EC10 could be related with the higher Mt induction levels which may confer larger tolerance and longevity to the particular stress. Further studies are ongoing.

ET10B-4
Global DNA methylation in Daphnia magna is influenced by genotype and a wide variety of environmental stressors
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Epigenetics is the study of mitotically or meiotically heritable changes in gene function that occur without a change in the DNA sequence. An important epigenetic control mechanism is DNA methylation, which was recently discovered in the water flea *Daphnia magna,* an ecotoxicological model species. It has been shown that exposure to relatively high concentrations of certain chemicals can alter the degree of global DNA methylation in *D. magna* and that this altered DNA methylation level is still found in two subsequent unexposed generations. Here we investigated the impact of a range of environmentally relevant stressors on global DNA methylation. *D. magna* of 14 days old from two different clones were exposed for 48 h to one the following stressors: chemical predation cues, strains of the cyanobacteria *Microcystis aeruginosa*, nutritional quality (essential fatty acids & sterol content), heat stress, metals (166 µg/L Pb, 6 µg/L Cd, 1000 µg/L arsenite) or salinity (5 g/L NaCl increase). All exposures were conducted in triplicate exposure aquaria, resulting in three biologically independent replicates per treatment. DNA was enzymatically digested to nucleosides (including 5-methyl-2'-deoxycytidine), which were analysed by UPLC-MS/MS. Overall, the global degree of cytosine methylation varied between 0.17 % and 2.54 %. An unexpected but interesting observation was the relatively high variation in global DNA methylation level between triplicates of some treatments, with relative standard deviations (RSDs) up to 71%. The extent of this variation between replicates differed between the various stress exposures, with fish predation cues inducing the largest RSD for both clones. Significant differences in global DNA methylation between treatments were observed. For instance we found 0.83% cytosine methylation in clone Xinb3 daphnids exposed to *Triops* medium, which is significantly higher than 0.35% and 0.44% in Xinb3 daphnids exposed to salinity or microcystin-producing *Microcystis*, respectively. Finally, differences between the two clones were observed, with global DNA methylation in the Xinb3 clone generally higher than in the Iinb1 clone, except for daphnids exposed to higher temperature. In conclusion, this study demonstrated that global DNA methylation in *D. magna* is dependent on genetic (clone) and environmental (exposure) factors. Further research is aimed at addressing the biological function of DNA methylation in *D. magna* and its potential long-term impact.

ET10B-5
Micropollutants' induced trait evolution in natural phytoplankton communities
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Evolution is a fundamental part of ecological theory and ecology and evolution together determine the dynamics of all living systems. Rapid and strong contemporary evolution can occur due to imposed selection, anthropogenic activity and environmental change including pollution. Trait-based approaches focus on phenotypic traits as the fundamental unit to understand adaptation in natural populations. Advances in scanning flow-cytometry afford high precision measurement of individually expressed phenotypic descriptors in phytoplankton. We report of natural phytoplankton communities exposed to micropollutants in the field. Effects were assessed as changes in mean phenotypic traits per population over a period of 7 generations post initial pulse exposure. Effects were also scaled to community level and shifts in trait-distributions compared to expected patterns from eco-evolutionary theory of community assembly. We tested the effects of triclosan, a common bactericide used in personal care products and routinely found in surface water, as a model micropollutant. We exposed natural lake phytoplankton communities to 0.1, 1 and 10 µg/L of triclosan, covering the range of environmental concentrations, in a novel type of permeable microcosms. These allow the exchange of nutrients and physical conditions with the external environment, while confining plankton and pollutants inside. At generation 7 post-exposure, both phytoplankton diversity and density responded to initial dosing of triclosan with a humped shaped "hormetic"relationship. We found that phenotypic traits associated to cell size and pigment content were significantly under selection in communites exposed to triclosan. We detected statistically significant shifts in the mean and range of size-related trait distributions, suggesting an "environmental filtering"effect. The rates of phenotypic change within phytoplankton populations showed to be significantly impacted by triclosan compared to control levels. The rate of size evolution was higher in treatments, while the rate of change in pigment content appeared to be reduced by the effect of triclosan compared to controls. We provide original findings that advocate for a more careful consideration of sub-lethal doses and environmentally relevant exposure scenarios in the risk assessment of chemicals, since evolutionary processes can occur at pulse exposure to low levels of anthropogenic chemicals in natural and complex communities.

ET11 - Marine environmental chemistry and ecotoxicology

ET11A-1
Using an integrated assessment framework for contaminants and biological effects to determine good environmental status under the Marine Strategy Framework Directive
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The use of biological effects tools offer enormous potential to meet the challenges outlined by the European Union Marine Strategy Framework Directive (MSFD) whereby Member States are required to develop a robust set of tools for defining eleven qualitative descriptors of Good Environmental Status (GES), such as demonstrating that "Concentrations of contaminants are at levels not giving rise to pollution effects"(GES Descriptor 8). Here we discuss the combined approach of monitoring chemical contaminant levels, along-side biological effect measurements relating to the effect of pollutants, for undertaking assessments of GES across European marine regions. Using data collected as part of the UK's Clean Seas Environmental Monitoring Programme (CSEMP) we outline the minimum standards that biological effects tools should meet if they are to be used for defining GES in relation to Descriptor 8. Adopting the recommendations of the ICES Study Group for the Integrated Monitoring of Contaminants and Biological Effects (SGIMC) we present a case study demonstrating how such an approach, using contaminant (e.g. metals, PAHs, PCBs) and biological effects (e.g. EROD, bile and pathology) data, in sediment, water and biota could be use used to determine GES in the marine environment.

ET11A-2
PAH and biomarker measurements in fish from condition monitoring in Norwegian waters from 2005 to 2011
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Condition monitoring in fish from open seas are performed in Norway every third year. The objectives have been to investigate whether fish from Norwegian seas contain elevated levels of components that originate from discharges from offshore oil and gas production. Focus has been on the Tampen region, as this is the region with highest discharges of produced water. In 2010, 128 mill ton produced water were discharged in the Norwegian sector of the North Sea, and 59 % (76 mill ton) were discharged at the Tampen region. Condition monitoring in 2002 demonstrated changed levels of several parameters in haddock from Tampen, compared with haddock from the Egersund Bank, including 2-4 ring PAH metabolites in bile, DNA adducts in liver, and the ratio of n-3/n-6 in muscle (Balk et al., 2011).
These results were followed up in 2005 and 2008. The main focus has been the North Sea (Tampen and the Egersund bank), but samples from the Norwegian Sea and the Barents Sea (reference area) were also analysed for comparison.
NPD and PAH measured in fish muscle and liver from cod and haddock in 2005 and 2008 were found to be below LOQ for all regions. The main contributor to sum PAH metabolites in bile at Tampen and at the Egersund bank measured in 2008 was 1-hydroxy phenanthrene. Levels of alkylphenols in bile were below LOQ. Levels of Vtg in blood of male cod were generally low from all regions.
DNA adducts in haddock liver were significantly higher at Tampen compared with Egersund Bank in 2005 and 2008, but to a lesser extent (2-fold in 2005 and 2008, compared to 5-fold in 2002).
Lipid content in the liver was significantly reduced in haddock from Tampen in 2008. Fatty acid profiles showed that haddock from Tampen had relatively high levels of arachidonic acid, and the ratio between omega-3 and omega-6 (n-3)/(n-6) poly unsaturated fatty acids were significantly lower in the lipid classes in haddock from the other regions.
Cod and haddock were also collected in 2011 and analyses are under process.
Reference
Balk L et al., 2011. PLoS ONE, Volume: 6(5), article no: e19735.
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ET11A-3
Biomarkers in marine mussels (Mytilus edulis) exposed to environmental concentrations of oil field corrosion inhibitors
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Biomarker assays were applied to the marine mussel Mytilus edulis exposed for 24 hours to environmental concentrations ranged from 0.001 to 1.0 mg/l of Produced Water (PW), Quaternary Ammonium Salts (QUATs), Imidazoline (IMD)and Phosphate ester (PE), all commonly used oilfield chemicals. The Comet assay was used to determine DNA damage in individual haemocytes and gill cells; furthermore Neutral Red Retention (NRR) was also used to determine the lysosom membrane stability (LMS), also a suite of oxidative stress endpoints, TBARS and Superoxide dismutase (SOD) activity. On the other hand, the mass spectrometry was used to assess the body burden of PW, QUATs, Imidazoline and phosphate ester. The result showed a significant increase in DNA damage in haemocytes and gill cells, as well as TBARS in gill cells at all QUATs, IMD, PE and PW concentrations. Lysosom membrane damage was significant increase at concentration ≥ 0.5 mg/l of QUATs and ≥ 0.3 mg/l of IMD. Moreover, SOD activity was significantly increased at concentrations > 0.01 mg/l of QUATs and at concentrations ≥0.001 mg/l of IMD, PW and PE at concentration ≥ 0.01 mg/l . These results may have consequences for offshore disposal of produced water.

ET11A-4
Accumulation of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in deep-sea biota and sediments from a submarine canyon (NW Mediterranean)
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Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) are a family of persistent organic pollutants (POPs) consisting of 210 possible congeners. Seventeen of these congeners (2,3,7,8-substituted) have been described as the most toxic. Submarine canyons are particular environments characterized by a fast transport of sedimentary material down slope resulting in an efficient mobilization of particle-bound contaminants from surface waters to deep-sea. No data has been reported on PCDD/Fs in biotic or abiotic compartments of submarine canyons to best of our knowledge. The objectives of the present study are: (1) to provide with the first assessment of PCDD/F levels in deep-sea crustaceans and fish as well as sediments in a submarine canyon (NW Mediterranean Sea); (2) to investigate the dioxin accumulation in crustacean and fish species from different water column's zones in relation to their habits (pelagic, nektobenthic and benthic). Higher Σ2,3,7,8-PCDD/Fs levels were found in general in crustaceans ranging from 220 to795 pg g-1 lipid weight (l.w.) (13 - 90 WHO98-TEQ pg g-1 l.w.), whereas levels in fish varied from 110 to 300 pg g-1 l.w. (22 - 33 WHO98-TEQ pg g-1 l.w.). Nektobenthic species (fish and crustaceans) exhibited the highest dioxin levels in the Blanes submarine canyon and adjacent open slope (in agreement with trophic chain studies that ranked nektobenthic organisms at the highest trophic level). Dioxin concentration in sediment were generally in the range of those reported for surface sediments in the Mediterranean sea coastal areas and in general in low to moderate anthropogenic impacted aquatic ecosystems world-wide. Differences in congener-specific accumulation were found between the pelagic crustacean and the benthic and nektobenthic crustaceans. Therefore, species habits seem to have an influence in the differential congener-specific accumulation of PCDD/Fs. A combination of factors such as biota habits, differential uptake of water column dioxin (dissolved and particle-bound fractions), dioxin pattern in the sediment and different metabolization capabilities and rates (CYP-mediated metabolism) can most probably explain the differences observed between crustacean species and between fish and crustaceans in the Blanes submarine canyon. However, further research is needed in order to evaluate the relative predominance of individual factors.

ET11A-5
Echinoderm early life development bioassays reveal effects of marine POP's
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Persistent organic pollutants (POPs) tend to accumulate in the food web reaching concentrations that potentially cause toxic effects. Early life stages (ELS) of vertebrates as well as invertebrates are regarded as the most sensitive to toxic effects of POPs. In addition several vertebrate species (e.g. amphibians, flatfish) comprise a thyroid hormone (TH) dependent metamorphosis which is known to be sensitive to thyroid hormone disrupting compounds (TDCs). The TH-regulated metamorphosis of echinoids (Echinodermata: Echinoidea), such as sea urchins, also is potentially susceptible to the effects of TDCs. In our study we have developed a prolonged ELS bioassay and a metamorphosis assay with the sea urchin Psammechinus miliaris. In addition a short term bioassay was developed to study the impact of marine POPs on the multixenobiotic resistance (MXR) mechanism. In this mechanism cellular efflux transporters form an important first line of defense against xenobiotics.
P. miliaris early life development was rather sensitive to HBCD (EC_{50} 54 nM) with a remarkably steep dose-response curve. The most sensitive endpoint to quantify adverse effects on development was the 'penalty points' approach determined at 16 days post fertilization. This delayed development is an important sub-lethal effect that cannot be determined in common echinoid ELS tests since the observation period ends between 48 and 72h post fertilization. TCS was acutely toxic during embryo development (≥ 500 nM) and at concentrations ≥1000 nM no hatching occurred. In contrast to the high sensitivity of amphibians and fish, P. miliaris early life development was not sensitive to dioxin-like toxicity (NOEC > 0.03 nM TCDD-equivalents (TEQs)). In the newly developed metamorphosis assay thyroxine (EC_{50} 0.09 nM) as well as an environmentally relevant PBDE mixture (EC_{50} 219 nM) accelerated metamorphosis, while TBBPA delayed it (IC_{50} 97 nM). Compounds tested in the cellular efflux transporter inhibition assay showed a clear dose related inhibition within 90 min. of exposure. The most potent compound was TCS (EC50 1 µM), followed by PFOS (EC_{50} 5 µM) and BPA (EC_{50} 146 µM).
The development of echinoids as an animal model for marine ecotoxicological studies is of great ecological relevance, and in addition it may contribute to the ethically desired reduction of the number of vertebrates currently used for testing endocrine disruption and teratogenicity.

ET11A-6
Species sensitivity distributions for ocean acidification effects
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Climate change can have large impacts on marine ecosystems via ocean acidification. Already for decades it is recognized that a continuous increase in greenhouse gas emissions leads to increased uptake of carbon dioxide into the oceans and possible ocean acidification that can affect marine species. The overall impact on marine ecosystems of ocean acidification is, however, not quantified before. The goal of our work is to assess the effect of ocean acidification on marine species groups via Species Sensitivity Distributions (SSDs). SSDs are commonly used to derive the proportion of species affected at different exposure concentrations of toxic pollutants. In this case, SSDs for ocean acidification express the cumulative fraction of species affected (PAF) as a function of pH in the marine environment. The concentration chosen as critical for a specific species is the pH value at which 50% of the population is affected (defined as EC50). To derive EC50 values, data was extracted from over 100 papers, covering over 100 marine species. SSDs are established for different species groups. Depending on the species group considered the SSD largely differs. First preliminary results indicate that when considering all species, around 15% of the marine species will be affected if the current pH drops to 7.8. The results of our work can be used within risk assessment to incorporate the effects of climate change on marine species via ocean acidification, next to the effects of other pollutants that are already considered.

ET11B-1

Automatic monitoring of chemical pollution in marine water: opportunities for cost effective solutions

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The European Union has recently established the Marine Strategy Framework Directive (MSFD, 2008/56/EC).The directive foresees the introduction of Environmental Quality Standards (EQS) in territorial waters (roughly 12 nautical miles form the coast line) for a range of chemicals, with particular focus on organic contaminants. Signatory countries are thus required by law to gain adequate infrastructures and tools to perform monitoring and demonstrate fulfillment of the standards. Measurements of organic micropollutants in marine surface waters are challenging and expensive, and often require high logistic costs, for example in relationship to ship hiring and mooring in deep waters.

We present here a new device which allow fully automatic monitoring of priority chemicals using regular line ferries or other fixed stations such as, for example, off-shore platforms. The system allows spatial and temporal integration of the monitoring data, and can be controlled from any computer or mobile device in any part of the world through internet and satellite communications. In addition, the sampler provides automatic procedures for preserving the samples after the collection is completed.

Sampling performance is currently under testing both on land based marine stations and on board of a ferry. We present here, in details, the features of the new device and the preliminary results from the proof of concept phase.

ET11B-2

Using ecotoxicological information in an integrative framework for the objective classification of ecological status of marine water bodies

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The European Water Framework Directive (WFD) introduced a new philosophy in environmental management and an ambitious goal: achieving a "good ecological status"of all surface water bodies for 2015. The classification of ecological status relies on setting arbitrary, type-specific reference values for a closed list of biological and chemical quality elements, among which neither biomarkers nor bioassays are considered. Due to natural variability of the WFD-proposed biological quality indicators, multiple problems have surfaced as soon as comprehensive efforts to standardise and harmonise those indicators have started, which is delaying marine water body evaluations beyond the deadlines established in the Directive. As an example, the transitional water bodies of the Minho estuary, the main estuary in the NW Iberian Peninsula, have been evaluated by neither the Spanish nor the Portuguese authorities due to the inexistence of appropriate intercalibrated indices.

An alternative methodology for the classification of ecological status using multivariate analysis, is proposed and applicated to a pilot study only for the purpose of illustration of the method. The scale of the study was not designed for classification of ecological status of water bodies. This approach does not depend on the arbitrary definition of fixed reference values and ratio-to-reference boundary values, and it is based on the application of non-metric multidimensional scaling (MDS) and cluster analysis to multivariate data sets encompassing chemical, ecotoxicological (biomarkers, bioassays) and ecological (community indices) information. A multiyear data set was generated from the investigative monitoring of marine pollution in Ría de Vigo (NW Iberian Peninsula) from 2004 to 2006, conducted in collaboration with the Spanish Institute of Oceanography (IEO) and the University of the Bask Country (EHU), that included water and sediment chemistry, sediment toxicity assessed by the sea-urchin embryo test, mussel bioaccumulation, mussel physiology (SFG), and benthic macrofaunal richness and diversity. The application of multivariate analyses to a comprehensive ecotoxicological data-set allowed objetive and robust classification of sampling sites into discrete categories of ecological status in a higly productive coastal ecosystem. This approach allowed integration of biomarkers and bioassays in the evaluation of coastal and transitional waterbodies according to the objectives of the European WFD.

ET11B-3

Managing environmental risk in marine coastal systems: development of an innovative expert decision support system

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Following the EU Marine Strategy Framework Directive (MSFD), member States should attain / mantain / restore a Good Environmental Status (GES) in marine areas in the next years. Risk-based frameworks are useful to objectively assess environmental quality on the basis of both chemical and biological investigations. An innovative Expert System has been developed to support decision makers in managing marine coastal areas and, in particular, sediments.

The Expert Decision Support System integrates chemical data by considering the toxic pressure due to all contaminants in sediments: concentrations are compared to TELs (threshold effect levels) and a chemical risk index (ChemRI) is calculated applying an additive model to the pollutant mixture. Ecotoxicological high-level endpoints (i.e. survival and reproduction) are used to calculate the ecotoxicological risk index (EtoxRI), while sublethal parameters (i.e. biomarkers) are integrated into the biological stress index (BSI). With the aim to optimize resources, ecotoxicological test selection should follow a 2-tiers framework: where chemical concentrations overcome PEL (probable effect level) high level endpoints (i.e. survival and reproduction) on different model organisms should be evaluated, while if contaminants are in the range between TEL and PEL, sublethal parameters should be added to the ecotoxicological battery.

Finally the Sediment Risk Index (SedRI) is calculated by combining ChemRI and EtoxRI. SedRI ranges form 0 (no risk) to 1 (strong risk): comparing SedRI with specific thresholds it is possible to rank the risk level of the sediment and consequently to correctly manage it by planning opportune interventions (e.g. land-filling, remediation, re-use after dredging). SedRI values in the intervals 0.75-1.00 and 0.50-0.75 respectively indicate high and medium risk; SedRI values in the ranges 0.25-0.50 and 0.00-0.25 individuate mild risk and no risk.

The Expert Decision Support System has been applied to integrate data from the scientific bibliography about sediment quality in some estuarine areas of Spain. Sediments are classified in terms of both chemical contamination and ecotoxicity and recommended interventions are indicated for each site.

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ET11B-4

Preliminary evaluation of a new tool for assessment of in situ biological exposure and effects in aquatic environments

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This paper summarizes recent activities associated with the development and demonstration of an integrated exposure and effects assessment approach involving multiple, concomitantly-linked in situ measurements towards improved ecological risk assessment. When conducted properly, in situ bioassays are a means of reducing uncertainty associated with traditional laboratory-based characterization of contaminated sediments and surface waters [1-3]. In situ exposures can increase realism through reduced sample manipulation and integration of a variety of site-specific factors, which affect contaminant bioavailability and toxicity. Incorporation of passive sampling devices (PSDs) into in situ studies has recently proven valuable as an additional line of evidence towards evaluating contaminant bioavailability [4]. In some situations, in situ characterization of sites is the only relevant means of accurately assessing exposure and effects. These scenarios include assessment of (1) in situ-based sediment remedy (e.g. reactive amendment) effectiveness; and (2) time varying stressors (e.g., storm water discharges, tidally influenced groundwater seepage, underwater unexploded ordnance/ discarded military munitions, and oil spills).

The Sediment Ecotoxicity Assessment Ring (SEA Ring) is a recently developed, field deployed device capable of housing an array of in situ bioassay chambers, PSDs, and water quality sensors. Exposure chambers are oriented such that various exposure routes (e.g. surficial sediment, sediment-water interface, water column) can be investigated. A research prototype SEA Ring has been successfully deployed and recovered at marine, estuarine, and freshwater sites varying in water depth and hydrology. These studies have largely incorporated minor modifications to standard laboratory methods using common test species [5,6].

The prototype SEA Ring is currently undergoing a series of refinements to standardize/control in situ exposure conditions, increase user-friendliness, and reduce dependence on divers. An overview of recent use of the SEA Ring, status of refinements to the device/approach, initial validation of new prototype performance, and discussion of efforts towards regulatory acceptance of the approach will be presented.

ET11B-5

DGT-copper flux measurements predict bioaccumulation and toxicity to bivalves in sediments with varying properties

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Many regulatory frameworks for sediment quality assessment include consideration of contaminant bioavailability. Analyses of pore waters or acid-volatile sulfide (AVS)-

simultaneously extractable metal (SEM) relationships frequently provide this information, but have significant limitations. Porewaters measurements are non time-integrated 'snap-shots'; acid-volatile sulfide (AVS) over-emphasises the importance of sulfidic sediments; and both these methods inadequately assess metal bioavailability for benthic organisms living in the surface layer (i.e. majority of organisms). The use of inappropriate or inadequate information for assessing metal bioavailability in sediments may result in incorrect assessment decisions. The technique of diffusive gradients in thin films (DGT) enables the in situ measurement of metal concentrations in waters and fluxes from sediment pore waters. Field deployments of this simple device can provide time-averaged information on site-specific in-situ metal fluxes from sediments to porewaters under the natural conditions. We believe these fluxes may provide the best measure of the 'release potential' of metals from sediments and thereby provide excellent information on metal bioavailability. We used the DGT technique to interpret the bioavailability of copper to the benthic bivalve Tellina deltoidalis in sediments of varying properties contaminated with copper-based antifouling paint particles. For a concentration series of copper-paint-contaminated sediment types (sandy, silty-sand and silty), DGT-probes were used to measure copper fluxes to the overlying water, at the sediment-water interface and in deeper sediments. The DGT-Cu fluxes were shown to provide excellent relationships with both the copper concentrations and properties of the sediments which influenced the bioavailability. Strong dose-response relationships were observed when DGT-copper flux was used to predict the bioaccumulation and lethal effects occurring to the copper-sensitive benthic bivalve, T. deltoidalis. The study demonstrates the strength of the DGT technique, which we expect will become frequently used for assessing metal bioavailability in sediments.

ET11B-6

Imposex in the dogwhelk (Nucella lapillus): monitoring around England and Wales since 1992

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Surveys of imposex in the dogwhelk (Nucella lapillus) have been conducted UK-wide on four occasions over the past eighteen years (1992, 1997/1998, 2004 and 2007) to assess the effectiveness of legislation controlling the use of TBT on yachts and ships in the marine environment. As a continuation of this assessment, a further survey was conducted between October 2010 and January 2011. Dogwhelks were collected from 76 sites around England and Wales and analysed for imposex (VDSI and RPSI) and data were assessed according to the criteria developed by OSPAR (Oslo Paris Commission). The results show a significant decline in the level of imposex. In 1992, 100% of sites showed VDSI values above 2, (OSPAR assessment class C) while in 2010/2011, only 10% of the sites showed VDSI values above 2 and 42% were below 0.3 (OSPAR assessment class A) . The data confirm that the legislation brought in by the International Maritime Organisation for large ships, and implemented during 2003- 2008, has been very effective in reducing the impact of TBT in the marine environment.

ET11C-1

Biomarker responses in the laboratory are confirmed in large scale field study of ten estuaries

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The use of biomarkers as a measure of ecological response to contamination is increasing in environmental monitoring programmes. Biomarker responses are complex and may be species-specific, therefore they require evaluation in species that are native to the bioregion being monitored before they can be applied by monitoring agencies. Using both laboratory experiments and a large scale field study, we validate the use of the Sydney rock oyster (Saccostrea glomerata) as a biomonitoring species, and assess the potential use of two cellular biomarkers; lipid peroxidation and lysosomal membrane stability. In the laboratory, oysters were exposed to various concentrations of Cu and Cd via either their food or surrounding environment (water) for 96-h. In the field, oysters were deployed for 3 months at 4-7 sites within ten estuaries in NSW with various degrees of human modification. Biomarker responses were measured in the digestive glands of oysters. In both laboratory and field experiments lysosomal destabilisation rates were significantly higher in systems with higher contaminant concentrations when compared with controls or cleaner reference sites. In the field study, lysosomal membrane stability was significantly related to individual metal (Cu, Pb and Zn) contaminant concentrations in suspended sediments. Lipid peroxidation concentrations were generally unaffected by contaminant exposures, with the exception of a low dose food-borne Cu (10 ug Cu L-1) exposure where lipid peroxidation concentrations were significantly increased. Lysosomal membrane stability in the Sydney rock oyster proved to be an efficient indicator of an organism under anthropogenic stress and these laboratory and field results further validate its use for studying biological effects of environmental pollutants in estuarine systems.

ET11C-2

Embryotoxic and genotoxic effects of pesticides and heavy metals on embryos of Pacific oyster (Crassostrea gigas)

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Although oyster embryos are used for decades to assess marine water quality, less is known about environmental pollutant genotoxicity and the link between genotoxicity and embryotoxicity in this sentinel species. The aim of this study is to assess embryotoxicity and genotoxicity of two dissolved metals copper sulfate and cadmium chloride (Cu, Cd) and two pesticides (metolachlor, irgarol) in Pacific oyster (Crassostrea gigas) larvae and to investigate the relationship between those two endpoints. Embryotoxicity was measured by calculating the percentage of abnormal D-shaped larvae and genotoxicity was evaluated with DNA strand breaks using the comet assay. After 24h-exposure, the percentage of abnormal D-larvae showed a significant increase from 0.1 µg L-1 for Cu ($p < 0.05$), 10 µg L-1 for Cd ($p < 0.05$) and 0.01 µg L-1 for both metolachlor and irgarol ($p < 0.001$) in comparison with the seawater control. Following 16h exposure, significant increases of DNA strand breaks were observed for both metals (from 0.1 µg L-1 and 10 µg L-1 for Cu and Cd respectively) and pesticides (from 0.01 µg L-1 for both metolachlor and irgarol) ($p < 0.05$). A strong positive relationship between embryotoxicity and genotoxicity was recorded for each tested toxicant ($p < 0.001$, $R^2 = 0.71$-0.88), except for irgarol showing moderated correlation ($p = 0.0019$, $R^2 = 0.57$). The current study suggests that some of these pollutants (irgarol, metolachlor and copper) can induce larval abnormalities and DNA damage in exposed oysters at environmentally relevant concentrations.

ET11C-3

First development and validation of genotoxicity biomarkers in frozen total blood of Xiphias gladius and Caretta caretta: a novel approach to evaluate genotoxic effects in wildlife

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The aim of this study was to develop and validate genotoxicity biomarkers (comet and diffusion assays) on frozen total blood in order to assess the DNA integrity in swordfish (*Xiphias gladius*) and the loggerhead turtle (*Caretta caretta*). Only a limited number of studies have been published that use these assays with these or other pelagic marine species. This might be due to the logistical problems associated with performing the techniques in open sea or in particular conditions. Moreover no authors have solved the fresh blood conservation problems over a long period of survey and none have assessed the biomarkers of genotoxicity on the cryopreserved blood of marine and freshwater fish species and reptiles. In the first phase of this work, a methodology using frozen conservation was developed in parallel with the comet assay on the fresh and cryopreserved blood of *Dicentrarchus labrax* and loggerhead turtles. In the second phase the techniques were validated on the blood of the loggerhead turtle and swordfish. No statistical differences were observed in the DNA fragmentation and apoptotic cells between the fresh and frozen blood of the *Dicentrarchus labrax* and loggerhead turtle. Regarding the comet assay, a positive correlation was found between the fresh and frozen blood of the loggerhead turtle. An age-related decrease in DNA fragmentation and an age-related decrease in apoptosis in the loggerhead turtle was observed. The female swordfish showed higher values of DNA damage ($p<0.05$) and lower values of apoptotic cells than males. This study enabled a whole blood cryoconservation protocol applied for the first time in an ecotoxicological investigation. This tecnique led to the avaluation of possible DNA integrity in two species never studied before in this field, such as the loggerhead turtle and swordfish. The findings strongly suggest that comet and diffusion assays in frozen blood can be used to assess genotoxic damage in a very high variety of species, from threatened species to species used for the environmental monitoring of remote areas. In addition, this innovative methodology could be carried out in all field sampling conditions and not only during laboratory experiments as has been the case until now.

ET11C-4

Temporal trend of organochlorine contamination in stranded and free-ranging striped dolphin (Stenella coeruleoalba) specimens in the Mediterranean Sea

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Mediterranean cetaceans are known to accumulate very high levels of xenobiotic contaminants, such as organochlorines (OCs). In addition they are susceptible to effects of these anthropogenic contaminants such as endocrine-disrupting effects, including consequences on reproduction and immunity. In 1990-1992, a severe Morbillivirus infection affected striped dolphins (*Stenella coeruleoalba*) along the Mediterranean coasts, starting from Spain and progressively reaching France, Italy, Greece and Turkey. In this period, the organochlorine levels in the blubber of stranded striped dolphin specimens resulted statistically higher than the levels of all other periods and the PCB concentration exceeded those estimated to be a threshold level in aquatic mammals for observed effects on reproduction and immunity. However, the precise roles of these xenobiotic contaminants in the epizoosis are unknown. Several hypothesis were formulated in order to explore the potential link between high OC levels and *Morbillivirus* infection. The aim of this work is to investigate the temporal trend of the levels of certain organochlorine xenobiotics: dicholorodipenyltrichloroethane (DDT) and its metabolites and polychlorinated biphenyls (PCBs) in subcutaneous blubber of free-ranging and stranded striped dolphin specimens sampled in the Mediterranean area from

1988 to 2011, especially considering that a new *Morbillivirus* infection is present in the Mediterranean Sea. In fact, it has been isolated a new virus strain closely related to the dolphin *Morbillivirus* that was isolated during the previous epizootic in 1990. The levels of organochlorine contaminants result always statistically higher in stranded specimens than in free-ranging specimens. Temporal trends of DDTs and PCBs of both stranded and free-ranging striped dolphins highlight that the alleged decline of these xenobiotic compounds, expected after the ban of DDT in the late '70s and from the PCBs regulation, is not detectable by our results. In fact, the decrease between the years 1996-2003 is followed by an increase of these xenobiotics with accumulation peaks in the specimens sampled in 2005. Hence in the future, along with studies that should be conducted in these marine mammals to assess the presence and toxicity of the new generation contaminants, such as flame retardants, should not be left out of investigation these 'dinosaurs' of the environmental contamination of xenobiotic origin.

ET11C-5
Organochlorine and polybrominated diphenylether compounds in the northern Baltic Sea food web
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This study explored PCDD/Fs, PCBs; non-dioxin-like PCBs and dioxin-like-PCBs (DL-PCBs), and PBDEs in the Baltic Sea fish; Baltic herring (Clupea harengus), salmon (Salmo salar), sprat (Sprattus sprattus), whitefish (Coregonus lavaretus), and vendace (C. albula), and their predator Baltic grey seal (Halichoerus grypus). Contaminant profiles demonstrate possible connections in the food web expressing intense predator-prey relationship. The sampling area covered the Gulf of Bothnia in the time period 2002-2007. PCDD/F sums and WHO-PCDD/F-TEQs were highest in salmon and herring. PCDD/Fs in grey seal existed general in low level. PCDD/F concentration in herring clearly exceeded the PCDD/Fs in grey seal although herring is the most important prey species of grey seal. The most abundant PCDD/F congeners in fish were 23478-PeCDF and 2378-TCDF. PCDD/F profile in grey seal was different expressing high contribution of 123678-HxCDD and OCDD. The highest PCB sum was detected in grey seal, followed by salmon and herring. A same trend was seen when considering a sum of 6 indicator-PCB congeners (PCB-28/31, -52, -101, -138, -153 and -180) of which -138, -153 and -180 were the most dominating ones in grey seal. WHO-PCB-TEQ was in the highest level in salmon, which indicates effective accumulation of toxic PCB congeners in salmon: The sum of the most dioxin-like non-ortho PCBs was overwhelmingly highest in salmon that affects straight to the WHO-TEQ level seen. Also mono-ortho-PCB sum was highest in salmon. PCB (non-dioxin-like PCB and mono-ortho-PCB) profile was very similar between the fish species and also between fish and grey seal. The profiles of 6 indicator-PCBs were similar in fish species, deviating slightly from grey seal. Non-ortho-PCB profiles were similar between the fish species, when grey seal had clearly more PCB-169 and less PCB-77 than fish. Salmon had the highest PBDE level, although grey seal was almost had equal PBDE concentration. The others stayed quite far below of these two species. BDE-47 was the most dominating PBDE congener in all studied species: quantitatively its amounts were biggest in salmon (3.8 pg/g ww) and grey seal; especially grey seal from the Bothnian Bay (9.2 pg/g ww). BDE-209, analyzed only from grey seal, did not contribute significantly to the overall PBDE cluster. Toxic load (WHO-TEQ) of grey seal probably greatly results from preferring herring and perhaps salmon in the diet, especially in the Bothnian Bay.

ET11C-6
PCDD/Fs and DL-PCB levels in Seagull eggs from natural and National Parks of Spain
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Polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans were studied in Yellow-legged gull (*Larus michahellis*) fresh eggs from several Spanish Natural or National Parks. These areas represent the most important gull colonies of the Iberian Peninsula and all of them are declared as Special Protection Area for birds (SPAs). Concentration levels for PCDDs and PCDFs ranged between 3-10 pg WHO-TEQ/g-lw. For DL-PCBs, high levels were observed with values ranging from 66 to 400 pg WHO-TEQ/g-lw. No significant differences were observed between the concentrations found in the samples collected during the two year sampling campaigns (2010-2011). In addition, Audouin gull eggs (*Larus auduinii*) collected from the Ebro Delta Natural Park were analysed and the results were compared with those obtained in the Yellow-legged gulls (*Larus michahellis*) eggs.. The high PCDD/F and DL-PCB levels found in the Audouin gull eggs were attributed to different feeding habits among species and it confirms that the gull diet has an important role in the accumulation of dioxins and related compounds.

ET12 - Soil ecotoxicology

ET12A-1
Will the ecosystem services concept help to tackle multiple threats to soil?
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Dutch soil policy are investigating how to facilitate optimal use of ecosystem services by land users and local authorities, in order to achieve more sustainable land use. The Dutch Soil Protection Committee (TCB) is preparing a scientific opinion to support this investigation.
Until now, soil protection was based on regulations for a number of management practices. The question is whether the ecosystem services concept will be able to provide better protection of soils than regulation and if it will allow to reduce the number of regulations, which is a political wish.
Using the ecosystem services concept to achieve more sustainable land use changes the approaches followed so far drastically. Ecosystems and the services provided by these are placed central in the concept instead of an environmental compartment (soil) or a threat (contamination). Therefore the use of the ecosystem services concept in environmental polices may in theory lead to integration of separate policies, over environmental compartments and over multiple threats. Another important aspect of the concept is that it focuses on the benefit people obtain from ecosystems. It is expected that the use of ecosystem services-language will motivate stakeholders better to use soil in a sustainable way, because it is for their own benefit.
Optimization of particular ecosystem services is considered to be more sustainable when the trade off against other services is limited as much as possible. Guidelines for the sustainable use of ecosystem services and for balancing benefits of optimising one service against the effects this may have on other services, have been described.
The ecosystem services provided by soils are mainly supporting services that contribute to the provision of final ecosystem services of which mankind directly benefits.
The maintenance of intermediate services may be seen as sustainable land use and is of crucial importance for the provision of final services. It seems plausible that this relationship works also the other way: overexploiting final services may lead to lesser quality of intermediate services. It is concluded that optimal use of ecosystem services may lead to sustainable land use, provided that the ultimate goal for 'optimal use of ecosystem services' is clear and guidelines are respected. This approach has the potential to address multiple threats, as mentioned in the European Soil Strategy.

ET12A-2
Sustainable risk-based land management on polluted sites
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There is an increasing request for biomass for biofuel production. However, the use of land to produce such biomass has raised a range of concerns about the sacrifice of food growing land, food security, food poverty and habitat conservation issues. In parallel brownfields exist for which there is no economic urge for restoration to conventional functional re-use and/or no realistic prospect for 'hard"re-use. The combination of biomass cultivation and soil rehabilitation could provide leverage to bring such degraded land back into use. Benefits from this kind of land use might include providing: a self-funding land management regime, economic activity to deprived areas, a long term improvement in land values and environmental benefits.
The project Rejuvenate started as a literature and interview study regarding advantages and disadvantages of non food biomass crop on marginal, especially contaminated, land and a decision support tool (DST) for biomass production on marginal contaminated land was developed. In the second phase case studies are performed which focuses on ecological risk assessments, environmental impact assessments and assessments of economical and social aspects for the demonstration sites in Sweden and Romania.
Test work has begun at one of the sites, where a site specific ecological risk assessment (Triad) was performed prior to cultivation and the addition of soil amendments and will be executed again during the project in order to gain insight in the effect of soil tillage and the addition of soil amendments. At all other sites chemical analyses of soil (including available fractions of pollutants) were performed, prior to cultivation and the addition of soil amendments and during the project. In addition chemical analyses are performed on different plants that are cultivated at the sites. The availability and uptake of pollutants in plants at treated and non treated soil plots will be compared in order to assess the effects (both positive and negative) of soil amendments.
This summer the analyses after one year of crop growth have been performed. The results will be presented at the meeting. At one site in Sweden a sewage sludge treated plot was compared with a non treated plot. At the sewage sludge treated plots the uptake of cadmium by plants (Salix) is decreased. The uptake of all other metals is in the same

order of magnitude in both test plots. The results of the other sites will be interpreted during the months to come.

ET12A-3

From single-species laboratory toxicity tests to assessing effects on soil biodiversity: how far can we jump?

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This contribution will review developments in soil ecotoxicology, emphasizing currently available tools and their potential to predict effects on soil biodiversity and soil ecosystem functioning.
In 1984 the first short-term toxicity tests with earthworms and plants were standardized, while tests were already available for determining effects on microbial processes. By early 2000 toxicity tests using sublethal endpoints were standardized for terrestrial plants, enchytraeids, earthworms, Collembola and predatory mites, and new tests developed for soil microorganisms. For earthworms and enchytraeids, avoidance behaviour tests and a bioaccumulation test have been described.
Most standard toxicity tests use single species, except for the ones on soil microorganisms. To assess toxic effects in more realistic settings, micro-ecosystems were developed to allow quantifying effects at the community level, taking into account species interactions. A field test is available for assessing pesticide effects on earthworms that can be combined with a litter bag test.
For reasons of standardization, most toxicity tests with soil invertebrates use artificial soil, or LUFA 2.2 soil as a suitable and natural alternative.
The quest for "putting more eco into ecotoxicology"triggered the focus on more ecologically relevant test designs, integrated approaches with responses at different levels of biological organization, taking into account the normal operating range of ecosystems. More complex issues receive attention, including ecological vulnerability, trait-based analysis and effects on functional endpoints (ecosystem services).
Many of the tests developed for assessing the toxicity of single chemicals are also used for assessing contaminated land, together with chemical analysis and field observations (TRIAD approach). To support field observations and monitoring, sampling methods for soil invertebrates have been standardized. Interpreting results of field sampling is hampered by difficulties to find suitable references. Sampling efforts like the Dutch Biological Soil Quality network may provide useful reference data.
Properly predicting effects on ecosystem structure and functioning in field soils will require moving to more complex test systems and further development of trait-based approaches and the ecosystem vulnerability concept. It will also ask attention for long-term effects of low exposures to persistent pollutants, possibly in combination with other stress factors.

ET12A-4

The use of a small-scale terrestrial ecosystem to evaluate pesticide mixtures

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Most studies in soil ecotoxicology evaluate the effects of one chemical in one test species using a reference soil, thus here is a need for a more comprehensive, holistic, hierarchical and integrated approach to assess the impacts of chemical pollutants on soil ecosystems. An attempt to accomplish higher "realism"in ecotoxicity evaluation was achieved through a higher tier experiment, using a small-scale terrestrial ecosystem ("STEM") containing soil from an agricultural field. Effects of the herbicide glyphosate, the insecticide dimethoate and the acaricide spirodiclofen to soil non-target organisms were evaluated using three concentrations: the field dose, i.e. recommended application dose, 5 and 10 times the field dose. The concentrations were chosen based on the predicted environmental concentration (PEC) according to the FOCUS model for each pesticide. Pesticides were applied at the soil surface, and effects were observed after a 28 days of exposure. Earthworms (Eisenia andrei), isopods (Porcellionides pruinosus), turnip seeds (Brassica rapa) and bait-lamina strips were used to survey the effects in single and binary combination exposures of the three pesticides. Results of the individual and binary exposures are discussed taking in account pesticide dissipation along exposure period and effects mainly driven by pesticide or due to interactions occurring in the soil system. In conclusion, the work made with the STEM provided a good insight about the effects of pesticide mixtures to non-target soil organisms, both in functional and structural endpoints, and permitted an evaluation of the interactions between organisms at different trophic levels after exposure to pesticides.

ET12A-5

Assessment of the interaction between Cu and temperature changes - a multispecies approach

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Temperature is an important aspect in the study of effects of climate change. Temperature may e.g. affect the reproduction, growth or survival of species. If combined with pollution, temperature effects may be even more severe to ecosystems. In order to assess the effects of the temperature alone and in combination with copper pollution in soil population and its processes, a two factorial soil multi-species experiment was performed. Six species representatives of different functional groups were tested over three exposure durations. Feeding activity and OM breakdown were also assessed. A range of temperatures resembling the annual variation for Denmark was used. Results showed that the food-web was significantly affected by Cu pollution and temperature increase. Different species reacted very differently to the distinct treatments applied but the major changes in the abundance of species were in general, attributed to the first 28 days of exposure. Litterbags and bait laminas showed Cu and temperature effects on the OM breakdown.

ET12A-6

Evaluation of the risk for soil organisms under real conditions

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It has been questioned in the last years if the risk assessment of Plant Protection Products (PPP) in soil addresses properly the relationship between pesticide exposure and effects on soil organisms. Thus, it is necessary to develop a new approach especially regarding the estimation of environmental concentrations within the soil profile. A study was conducted in Terrestrial Model Ecosystems (TME) analysing on the one hand the effects of two model pesticides with different physico-chemical properties (Lindane, Imidacloprid) on soil organisms (collembolans, oribatid mites, enchytraeids, earthworms). On the other hand, the exposure of soil organisms, i.e., the pesticide movement was monitored in the soil profile over time. The pesticides were applied on 68 TMEs (‘ 467 mm, height 400 mm), in two concentrations each; untreated TMEs served as controls. Additional TMEs for chemical analyses, for soil moisture/temperature monitoring, and for quantification of percolation water were installed. Different soil layers (0-2,5 cm, 2,5-5 cm, 5-10 cm, 10-20 cm), were separately analysed and results are shown for soil arthropods (collembolans, oribatid mites) colonization and pesticide contents. The results of the present study enlighten the spatial and temporal pathway of pesticides along with the effects on soil organisms in different soil layers. This project will provide an insight into relevant issues for the risk assessment of PPP based on results under realistic environmental conditions.

ET12B-1

Monitoring microbial diversity in European soils: ongoing projects and challenges

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According to the Convention of Biological Diversity (Rio de Janeiro, 1992), biodiversity is the variation in life from genes to species, communities, ecosystems, and landscapes. Soils represent a huge reservoir of biodiversity which varies in terms of taxonomic richness, relative abundance and distribution according to soil types, climatic conditions, vegetation and land uses. The key functions supporting ecosystem services as identified in the Millennium Ecosystem Assessment (MEA, 2005) largely depend upon organisms that inhabit the soil. Thus, the design and implementation of a sustainable soil management strategy requires a better knowledge of soil biodiversity. Against this background,

soil biodiversity is subject to various threats. The most serious are caused by anthropogenic activities which can impair soil biodiversity and functioning with negative consequences on ecosystem service delivery, with consequent effects on primary production and soil sustainability. Determining the range of biodiversity and its impact on soil functioning and ecosystem services is therefore a critical challenge which needs to be addressed.
This talk will give a short overview, with a specific focus on microbial biodiversity, on national (France, The Netherlands, United Kingdom) and European (EcoFINDERS) initiatives which have been undertaken to assess variations of biodiversity at large spatial scales according to soil and climate types and land uses. The EcoFINDERS (Ecological Function and Biodiversity Indicators in European Soils) project supported by the European Commission was launched in order to gain information on soil biodiversity (including both microorganisms and fauna). This project will result (i) at the scientific level in increasing our knowledge of soil biodiversity and its role in ecosystem services across different soils, climate types and land uses, (ii) at the technological level in the standardization of methods and operating procedures for characterizing soil biodiversity and functioning, and the development of bioindicators, (iii) at the economic level in the assessment of the added value brought by cost-effective bioindicators, and of cost effectiveness of alternative ecosystem service maintenance policies. The management of such large sampling schemes requires the development of platforms allowing the extraction, storage and analysis of the DNA, such as the GenoSol platform (www2.dijon.inra.fr/plateforme_genosol/).

ET12B-2
Evaluation of soil biodiversity in Germany - Compilation and analysis of soil status with regard to the implementation and advancement of the national strategy on biological diversity
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According to the German Law on Soil Protection (1998), one of the natural functions of the soil is its ability to act as a habitat for organisms (mainly invertebrates and microbes). So far, this function is not considered when assessing the quality of soils, e.g. in the context of landscape planning. Therefore, in a project supported by the German Federal Environmental Agency (UBA), the following issues were addressed:
- Critical compilation of existing concepts and approaches in the area of biological soil classification and assessment;
- Assembly of a data-base on soil biodiversity in Germany, in particular collection of data from German Permanent Soil Monitoring Sites (BDF) and from literature (Collembola, Oribatida, Lumbricidae, Enchytraeidae and microbes);
- Preparation of recommendations for the implementation of soil biological monitoring concepts and methods in Germany.

In this contribution, an overview on the results is given. After a short description of the data base (structure, content), the distribution of species in correlation to site conditions is presented. In addition, ecological profiles of relevant species from different groups have been prepared. Most importantly, the composition of communities (i.e. species assemblages) for different biotope types is shown. These are the first steps to develop reference values (= normal operating range of species and communities). Finally, recommendations concerning suitable organism groups and standard methods (including - mainly microbial - genetical indicators) for a nation-wide soil biodiversity monitoring program are given.

ET12B-3
Bacterial community composition and Cu tolerance across a soil pollution gradient: exploring the bacterial domain for Cu-responding taxa by pyrosequencing
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Toxic metals affect the composition and metal tolerance of microbial communities, but very little is known about specific bacterial taxa responding to Cu toxicity in soil. We used bar-coded pyrosequencing of the 16S rRNA gene to determine the long-term impact of copper (Cu) on bacterial community composition within a soil contamination gradient (20 - 3537 µg Cu g^{-1}) established by CuSO4 pollution more than 85 years ago. In parallel, we monitored microdiversity of culturable Pseudomonas spp. by universally primed PCR (UP-PCR) genotyping. Community composition were linked to pollution-induced community tolerance (PICT) data generated by the [^{3}H]leucine incorporation method and to detailed physicochemical characterization of the soil Cu gradient soils including analyses for bioavailable Cu ($[Cu]_{bio}$) by a whole-cell bacterial biosensor.
Relative abundances of the most abundant phyla (Actinobacteria, Proteobacteria and Acidobacteria) were not correlated with $[Cu]_{bio}$, but abundances of specific classes belonging to these phyla frequently correlated to $[Cu]_{bio}$. Relative abundances of several less abundant phyla (Bacteroidetes, Verrumicrobia, Cloroflexi, WS3, and Planctomycetes) were all negatively correlated with $[Cu]_{bio}$. The genus Ilumatobacter was abundant in low-Cu soils (2-3 % of all sequences), but its abundance was strongly decimated in the most contaminated soils. Remarkably, the number of unique operational taxonomic units (OTU's; 97 % similarity level) was not correlated to $[Cu]_{bio}$. Likewise, rarefaction analysis and calculated diversity indices did not indicate any trend between $[Cu]_{bio}$ and diversity. By contrast the microdiversity of culturable Pseudomonas spp. was negatively affected by Cu as witnessed from a reduced number of unique UP-PCR genotypes in High-Cu soil. Bacterial community tolerance to Cu was strongly correlated to all measured Cu exposure descriptors indicating that the community was indeed strongly selected by Cu. Principal component analysis performed at the taxonomic class level reinforced the role of Cu as selective agent as PC1 was strongly correlated ($P < 0.001$) to $[Cu]_{bio}$.
Our data demonstrates that Cu exposure selects for Cu tolerant bacterial communities with changed community composition, but unaltered "species"richness at the taxonomic resolution obtainable using 16S rRNA gene sequencing. In addition, we propose that the unexplored genus Ilumatobacter may represent a sensitive indicator group for soil Cu toxicity.

ET12B-4
Soil microbial biodiversity indicating adverse effects of pharmaceutical antibiotics
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Agricultural soils receive pharmaceutical antibiotics with contaminated manure used for fertilization. Many antibiotics, being bioactive agents, tend to persist in soil for month. Hence, it is expected that antibiotics adversely affect the functional and structural diversity of soil microbial communities. Vice versa, endpoints addressing biodiversity might be suitable for the ecotoxicological testing of antibiotics in soil. Results are presented on the effects of pharmaceutical antibiotics and especially sulfonamides on soil microorganisms, tested in microcosm, mesocosm and field experiments. Contaminated manure was used for soil spiking. Phospholipid fatty acids (PLFA) and 16S rRNA genes were analyzed as markers of the soil community structure and functional enzymatic activities were determined. Manure had a strong influence on functional and structural parameters, increasing the activity of selected enzymes, basal respiration, microbial biomass and PLFA parameters. Following introduction with manure, the extractable concentrations of antibiotics and especially the $CaCl_2$-extractable antibiotic fraction (defined as bioavailable) quickly dissipated in soil, while effects on microorganisms tended to increase on a mid-term. This led to an apparent concentration independence of antibiotic effects. Some of the effects of the sulfonamide SDZ on soil microorganisms were significant for several months and observed for up to 120 d. Effects of SDZ on single functional parameters were often small and insignificant. However, combining test results to functional patterns more clearly revealed effects and it became evident that key-functions, e.g. of the N-cycle, were adversely affected. Distinct effects of SDZ on microbial biomass and structural diversity were determined from PLFA and 16S rRNA gene analysis, which effects interacted with the manure application rate. Both manure and SDZ induced shifts in the community structure, making communities of different soils and soil compartments more similar. Derived ratios of bacteria-to-fungi and $gram^+$-to-$gram^-$ bacteria indicated changes in the structural diversity induced by SDZ. Furthermore, manure-borne bacteria, designated as antibiotic-resistant, survived several weeks in soil treated with SDZ contaminated manure as was shown by sequencing of selected bands.

ET12B-5
Using soil microarthropod community testing to increase ecological relevance of effect data in pesticide risk assessment
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In the present study, a new complementary approach combining the use of the natural soil microarthropod community and conventional test methods was used. The effects of soil contamination with the insecticide carbofuran on two geographically microarthropod communities (Warm Temperate and Tropical) were evaluated under in their soils of origincontrolled laboratory conditions.
After contamination of two agricultural soils from Portugal and Brazil, a gradient of concentrations was prepared. Soil cores were taken from the respective uncontaminated surrounding areas and the mesofauna of three cores was extracted directly to the test soil. After extracting the microarthropod communities to the test soil, these were incubated under laboratory conditions for 4 weeks, after which the mesofauna was extracted again. The organisms were assorted into higher taxonomic groups and Acari and

Collembola were respectively assorted into order/sub-order/cohort and family. Collembolans were still classified according to morphological traits and used as a case-study of trait-based risk assessment (TERA, Baird et al, 2008) of pesticides.
The exposure to insecticide contamination caused the impoverishment of the taxonomic diversity in both communities. Significant shifts in the microarthropod community structure in the different carbofuran treatments were found for the both soils, although effects were more pronounced in the assay performed with the soil from Brazil. Collembolans were the most affected group with a strong decline in their abundance. A dose response relationship was observed, showing a consistent decline on the relative abundance of Isotomidae, closely followed by an increase of Entomobryidae. Contrastingly, Acari (especially Oribatida) tended to increase their numbers with higher concentrations.
Trait-based analysis of Collembola data suggested that a shift in the functional composition of the communities occurred due to carbofuran soil contamination and that species adapted to deeper soil layers were more vulnerable to insecticide toxicity.

ET12B-6
Effects of insecticidal Cry proteins on nematodes - implications for the ecological risk assessment of a stacked Bt-maize variety (MON89034xMON88017)
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ET12C-1
Assessment of contaminated soil in the Canadian boreal forest using standardized toxicity tests
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There is an increasing need in Canada for soil toxicity test methods applicable to the boreal forest and northern regions to serve the needs of both industry and government for assessment and site remediation of thousands of industrial sites in these habitats. To meet this need, Environment Canada and the Saskatchewan Research Council are developing a suite of single-species terrestrial toxicity test methods. Ecologically-relevant boreal plant and soil invertebrate species were selected and brought into culture in the laboratory. Seeds were obtained from Canadian collections or suppliers or were field-collected while candidate invertebrate species were collected through heat-extraction of various boreal reference soils from across Canada. Growth of plants and survival and reproduction of invertebrates was measured in clean reference soils to establish performance measures. Test method development has focused on seven plant species, an oribatid mite species, two collembolan species and two earthworm species. A case study of toxicity testing of a soil impacted by weathered hydrocarbons will be presented and test results indicate that the use of a test battery of both plants and invertebrates was important for a complete assessment of eco-risk at the site

ET12C-2
Development of a microbial test method suite for assessment of contaminated soil toxicity to indigenous boreal forest microbial communities
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Environment Canada (EC) has developed test methods to evaluate soil contaminant toxicity to invertebrate and plant species in agronomic soils. As soil microorganisms play a significant role in nutrient cycling and organic matter decomposition, the need to develop a test method to assess the impact of soil contaminants on indigenous soil microbial community health is apparent. The boreal forest region of Canada accounts for over 50% of Canada's land mass. As such EC is currently working to develop suitable test methods, including soil microbial community health, to assess contaminated soils in this region. The proposed test method for the evaluation of soil contaminant toxicity to indigenous soil microbial communities in boreal forest regions incorporates a suite of tests, each with different endpoints, to reflect the reality that endemic soil microbial communities vary from site to site. The test suite evaluates soil microbial biomass, activity, and community structure using the following tests: fumigation-extraction, nitrification, ammonification, organic matter decomposition, substrate-induced respiration, basal respiration, bait lamina, enzyme assays, community level physiological profiling, and denaturing gradient gel electrophoresis. Results of freshly-collected reference and petroleum hydrocarbon contaminated boreal forest soil from Alberta, Canada will be presented.

ET12C-3
Ecotoxicological assessment of soils contaminated primarily with petroleum hydrocarbons (PHCs) and/or metals and salt
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In Canada, soils contaminated with petroleum hydrocarbons are managed on the basis of four hydrocarbon fractions. There are Tier 1 Canada-wide soil standards for each fraction designed to protect ecological receptors exposed via the direct contact exposure pathway. If these standards are exceeded by fraction-specific concentrations in the soil, then the proponent has the option to conduct a Tier 2 ecotoxicological assessment to demonstrate that 1) the exposure pathway can be excluded; 2) PHC residuals are stable and represent minimal risk to soil organisms; or 3) data generated can be used to derive site-specific remedial objective (SSROs). In practice, Tier 2 ecotoxicological assessments are conducted primarily to demonstrate that PHC residuals are stable and represent minimal risk to soil organisms. The data from the toxicity assessment must satisfy criteria established for different land uses classes. This is called the Tier 2 Pass/Fail approach. If the site passes, then no further remediation or action is required and site closure can be obtained when other lines of evidence corroborate minimal risk. However, if the site soils fail to satisfy the criteria, the proponent must select management alternatives to mitigate risk. Alternatively, the data generated from the Tier 2 ecotoxicity assessment can be used to derive site-specific remedial objectives to guide future remediation. The current challenge facing regulators, assessors, and managers alike is the lack of a framework or process for the derivation of these Tier 2 SSROs. A case study demonstrating the successful application of the Tier 2 Pass/Fail Approach will be presented. A case study where the approach failed will also be presented. A process that is scientifically defensible will be presented to demonstrate how to derive SSROs where the data signal to noise ratio is small.

ET12C-4
What does ecotoxicity testing tell us about dredged sediments?
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Research is focused on the actual environmental issue - a dredged sediment application on soils and preceding evaluation of their ecotoxicity to prevent possible ecological risks. High volumes of sediments are removed from rivers and ponds routinely. Although they may have a lot of positive properties (e.g. high nutrient levels) there are reasonable suspicions about contaminants present accumulated sediments. Therefore, an assessment scheme is necessary which helps to decide, if the land application will be allowed for every dredged sediment. This assessment cannot be based only on chemical analyses and should be accompanied by ecotoxicological testing. In the Czech Republic, novel directive 257/2009 Coll. for the dredged sediments application on agricultural land was ratified to determine the conditions when it is allowed to use sediment on agricultural soils. Innovation of this directive is the inclusion of four contact bioassays into the assessment. The most important question is, which bioassays should be included in the test batteries for the complex material ecotoxicity testing. The no-less important question is if the ecotoxicity bioassays reflect the contamination of the samples or their physico-chemical properties of other factors. The aim of this study was to answer the question about the appropriate battery of ecotoxicity bioassays for the dredged sediments testing and to investigate the driving factors affecting the results of the bioassays. Extended battery of traditional and new soil bioassays as well as tests of eluates was used for testing thirty-six different sediment samples.

ET12C-5
Eco-restoration of large-scale anthropogenic disturbances in the Boreal Shield: effects of moisture content on zinc availability and toxicity to the mite Oppia nitens and collembolan Folsomia candida
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Contamination of soil is often the most common form of anthropogenic stress in ecosystems and can pose threats to human health and the environment. Metal toxicity in soil ecosystems affects microbial, fungal and invertebrate populations. These effects are often compounded by other physical stressors like acidification and moisture stresses. The Flin Flon MB, Canada is a prime example of a boreal area experiencing forest dieback and a loss of plant biodiversity associated with mining and smelting activities for metal ores especially copper and zinc but also cadmium and lead. For many years some effort (mainly liming) has yielded moderate output on restoration in some areas, while other areas are recalcitrant. As part of the effort geared towards understanding the factors that might modulate metal toxicity and/or recovery in the area, we assessed the effect of moisture on Zn toxicity to two invertebrates with contrasting uptake routes but occupying similar trophic levels; the oribatid mite Oppia nitens and the collembolan

Folsomia candida. We singled out Zn after a relative to Cd toxicity model (TCd) proposed by Hopkin and Spurgeon (2001) identified Zn as the metal of most concern in the Flin Flon area. Initial trials to assess the effects of moisture on Zn toxicity using contaminated field soils (with or without organic matter adjustments) were not successful due to complicating effects of soil properties and low reproduction/survival of the invertebrates. We therefore used an artificial soil system and adjusted the moisture contents to 30, 45, 60 and 75% of the maximum water holding capacity of the soil. After four weeks, we assessed availability of the metals using an ion exchange resin membrane fractionation procedure and compared survival and reproduction as well as metal accumulation in the organisms. The results showed that with increased moisture level, there was increased availability of Zn in the substrates and increased accumulation in the mites. This was further confirmed by the result of life-cycle effect for both invertebrate species which showed increased Zn toxicity as moisture level increased. The results indicate that irrespective of invertebrate species involved, increased moisture had a surging effect on Zn toxicity, and therefore, methods aimed at keeping soil moisture low in remediated areas would be of importance in eco-restoration of contaminated sites. The implications in risk assessment of Zn in contaminated sites are discussed.

ET12C-6

Applicability of the Caenorhabditis elegans survival, growth and reproduction test to assess the effects of biosolids used in agriculture

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Modern high productivity agriculture has led to an impoverishment of nutrients and organic carbon in field soils, and as a consequence plants nutrition complements like fertilisers or wastes have to be used to keep high yield. These complex materials can also be a source of toxicants, depending on their origin, so there is a necessity to assess their environmental effects before field use. In this study, biosolids used in agriculture were tested as a mix with standard soil at different field dose through ecotoxicity test on the nematode *Caenorhabditis elegans*, endpoints for this organism being survival, growth and reproduction. In order to run these tests we first followed the standardized protocol for sediment and soil testing with *C. elegans* (ISO 10872, 2010). These results showed that the mixtures could be significantly different according to the type of soil (e.g. two phases system with overlaying water). Indeed, soil moistening in the standardized protocol is based on soil dry weight which means that the same amount of water is added for every soil, regardless of the water holding capacity (WHC) of the samples. Moreover, some biosolids can have a high WHC what increase humidity's differences among soil and soil mixed with biosolids when moistened as recommended by this protocol. To prevent this, improvements of the standardized protocol were developed, in which water addition is based on soil WHC instead of soil dry weight. Moreover, food volume added to the samples was lowered in order to be able to include it in the volume for soil moistening. In this context and in order to validate this new method, comparisons between standardized and optimized protocols were carried out for endpoints responses in five soils with different textures. This optimized protocol was used to assess the effect of different biosolids (limed sludge, manure ...) mixed with standard soil at different field rates and an example for a limed sludge is given. Regarding these results, improvements of the standardized protocol are adapted to assess the effects of these materials on *C. elegans*.

ET13 - Natural toxins and bioactive compounds

ET13A-1

Hydroxylated / Methoxylated analogs of Polybrominated Diphenyl ethers: biotransformation products of PBDEs or natural products

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Brominated compounds are ubiquitous in the aquatic environment. The polybrominated diphenyl ether (PBDE) flame retardants are anthropogenic compounds of concern. Results of some studies have suggested that PBDEs can be biotransformed to hydroxylated brominated diphenyl ethers (OH-BDE) and subsequently to methoxy BDE (MeO-BDE). However, the efficiency of transformation of PBDE to OH-BDE formation observed has been extremely small and could be accounted for by trace contaminants in the experimental materials. OH-BDEs have also been identified as natural compounds produced by some marine invertebrates. Another class of compounds, the methoxylated BDEs (MeO-BDEs), has also been identified as natural compounds in the marine environment. Both the OH-BDEs and MeO-BDEs bioaccumulate in higher marine organisms. Recent studies have demonstrated that MeO-BDEs can be biotransformed to OH-BDEs and this generates greater amounts of OH-BDEs than could be generated from PBDEs. Consequently, MeO-BDEs likely represent the primary source of metabolically derived OH-BDEs. Given that for some endpoints OH-BDEs often exhibit greater toxicity compared to PBDEs, it is prudent to consider OH-BDEs as chemicals of concern, despite their seemingly "natural"origins.

ET13A-2

Release and distribution of the bioactive compound artemisinin in the soil in and near Artemisia annua plantations

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Growing of biomedicine crops, i.e. bioactive compounds produced in plants and extracted for pharmaceutical products, require monocultures of plants with high contents of the desired bio-active molecules. Artemisinin is produced by *Artemisia annua* L. and used as active ingredient in anti-malaria drugs. Cultivation of *A. annua* in field scale implies high amounts of artemisinin produced and potential high losses to soil and leaching to the aquatic environment, where artemisinin could impact vulnerable organisms. Knowledge of the release routes of active compound from plant to soil, soil concentrations as well as within and around plantations is pivotal to risk assessments of biomedicine plantations.

To evaluate the release routes *A. annua* was grown in the green house, where leaf content, total soil content as well as contributions to total soil content from debris of dead leaves, rain off and root exudation of artemisinin were measured every second week over a full growing season. The dynamics of artemisinin in a field soil was investigated under Danish conditions measuring artemisinin in depth integrated samles (0-100 cm) and along a transect (0-20 m from the plantation). Artemisinin was measurable up to 15 ms away from the *A. Annua* plantation in the direction of the dominating wind. Large amounts of precipitation during the experiment facilitated transport of artemisinin to lower soil layers at the level of the drainage pipes. Hence, leaching of artemisinin to streams and lakes is a considerable risk.

Rain off contributed to the release of artemisinin to the soil environment, but the biggest contributor was dead leaves. Root exudation was a minor contributor. The amount of artemisinin in rain off followed the leaf concentration, whereas the total concentration in soil followed the amount released from dead leaves. The artemisinin content in soil reached steady state around week 21, suggesting a degradation rate in the similar magnitude as the input rate.

A model of inputs and outputs of artemisinin to the soil environment will the provided at the symposium, as a tool for risk assessment and risk managing when cultivating plants producing a bioactive compound.

ET13A-3

Production and emission of mycotoxins from a Fusarium infected winter wheat test field

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Mycotoxins are naturally occurring secondary metabolites of fungi colonizing a variety of cereals, fruits, vegetables and organic material in the soil, but can also arise due to moist conditions during storage. Mycotoxins have been studied intensively for decades due to their occurrence in food and feed and, hence, their potential threat to human and animal health.

Recently published data on two prominent mycotoxins, i.e., deoxynivalenol (DON) and zearalenone (ZON), indicates that the aquatic environment can also be exposed to mycotoxins. The identified main input sources of these mycotoxins into the aquatic environment included 1) run-off and drainage water from fields cultivated with cereals, like wheat or corn, 2) manure application and excretion from grazing livestock and 3) human excretion via sewer systems. This raises the question about the emission of other similar compounds from agricultural areas cropped with cereals. Therefore, the production of mycotoxins on a Fusarium infected winter wheat test field and their emission via drainage water was studied. Data from this campaign will be presented here.

ET13A-4

Risk assessment of Bacillus thuringiensis var. israelensis and spinosad on Polypedilum nubifer and Tanytarsus curticornis (Diptera: Chironomidae) in coastal wetlands

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Larvicides used for mosquito control are introduced into aquatic ecosystems where mosquito larvae develop (marshes, ponds, sanitation devices). A number of natural products have been proposed as 'environment-friendly' insecticides and some of them exhibit selectivity towards certain insect taxa which promotes their use for mosquito control. This is the case for the bacterial larvicide Bacillus thuringiensis var. israelensis (Bti), widely used for mosquito control all over the world, and for Spinosad, a mixture of spinosyns A and D known as fermentation products of a soil bacterium. Therefore, the present study was undertaken to assess the impact of Bti and spinosad on Mediterranean coastal wetland populations of Polypedilum nubifer (Skuse) and Tanytarsus curticornis Kieffer (Diptera: Chironomidae). Unlike Bti, spinosad had a strong lethal effect on P. nubifer population and seems to affect T. curticornis at presumed recommended rates for field application. But differences observed in term of sensibility

between this two chironomid populations confirm that the assemblage of the arthropod community as well as the population dynamic need to be known for the evaluation of risk of pesticides at the population level.

ET13A-5

New vectors of PSP, spirolides and okadaic acid in the North Atlantic: implications and impacts in marine ecossystems

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This work was done under the project ATLANTOX, aiming the discovery of emerging marine toxins in the Atlantic ocean, increased by the impact of anthropogenic inputs and climate change. For that purpose the Portuguese coast was surveyed between 2009 and 2010 for three distinct biotoxins (Saxitoxin group (PSP), Spirolides (SPX) and Okadaic Acid (OA)) in 15 benthic species of molluscs and echinoderms. These species where selected by their importance in the food-chain and to search for the presence of new vectors. The invertebrates were collected along the Portuguese coast since the summer of 2009 till the end of 2010.
For PSP's samples were analysed by the Lawrence method, for SPX LC-MS/MS techique was performed, and UPLC-MS/MS for OA analyses.
Results show that we are in presence of 16 first reports of these toxins in these endemic species: PSP's in G.umbilicalis, N.lapillus, Monodonta sp., P.lividus, M.glacialis and A.depilans; SPX in G.umbilicalis, N.lapillus, Monodonta sp., M.glacialis and P.intermedia; OA in G.umbilicalis, N.lapillus, Monodonta sp., P.lividus and M.glacialis. These results show that in the north Atlantic area new vectors of PSP, SPX and OA were found. The values obtained for some species such as the sea stars and the gastropod N.lapillus show that toxin transfer along food chain occur via mussels. New toxin routes especially including edible ones provide evidences that monitoring of marine toxins should be extended to other species than bivalves in order to prevent human health risks.

ET13A-6

Variations in the microcystin congener composition of lakes

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Cyanobacteria can produce a wide range of compounds which are toxic to other organisms including humans. The microcystins appear to be the most widely encountered cyanotoxins in freshwater and over 70 different types of congeners have been described. The drinking water standards are based on microcystin-LR and less attention has been given to other congeners. However, other microcystins and the actual composition observed in lakes should also be of interest since some microcystins are much more toxic than others. Unfortunately only about a dozen microcystins are commercially available and can thus be routinely quantified. At present, the factors that regulate the composition of microcystins in the environment are largely unknown. Culture studies and empirical observations of European lakes has suggested that light and nitrogen availability may influence the observed microcystin congener composition of lakes. Using ongoing water intake and lake monitoring data as well as literature data we examined the effect of lake trophic state on the relative abundance of microcystin congeners. Observations from lakes in central Canada (Ontario and Quebec) suggest that microcystin- LA, which has a similar mammalian toxicity as -LR, may be more common than in more nutrient enriched European lakes. Microcystin-LA concentrations appeared negatively correlated with microcystin-LR concentrations and the proportion of microcystins as microcystin-LA declined with increasing total microcystins.

ET13B-1

Trade-offs in herbicide-stressed Microcystis aeruginosa: growth vs. toxin production

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Organisms allocate assimilated resources into competing metabolic demands, such as maintenance, growth and defense. This pattern of allocation may change in response to stress and result in an increment of resources allocated to one trait and a decrement to another trait. The aim of the study was to test for the existence and magnitude of a potential trade-off between growth and toxin production in *Microcystis aeruginosa* exposed to the herbicide glyphosate at different light regimes. The ecological costs of microcystin production were estimated from differences between growth rates in a wild type strain of *M. aeruginosa* (PCC 7806) and its mutant unable to produce microcystins (PCC 7806 ΔmcyB). The trade-off (negative functional interaction between traits) triggered by glyphosate was only detected when cultures were incubated under photoperiods longer than 8 h of light (12:12 and 16:8), with different patterns for the microcystin analogues, time of incubation, and exposure concentration. Growth was the prioritized trait (ratio > 1), especially under the shortest (8 h) and longest photoperiods (16 h) during the first week of exposure. The magnitude of the trade-off ranged from 3-5[GREEKX] for the most toxic microcystin analogue MC-LR and and 2.5-12[GREEKX] for its demethylated variant [D-Asp3]MC-LR. The ecological costs involved in microcystin production were higher during the first week of incubation compared with the second. The mutant unable to produce microcystins had generally a higher growth rate than the wild type, regardless of presence or absence of glyphosate and especially during the first week of incubation. Glyphosate conferred additional ecological advantages to the mutants expressed in terms of tolerance and benefits to the wild type cultures during late exponential growth phase by either increasing toxin production or inhibiting the extracellular releases to the medium. The negative production rates calculated for [D-Asp3]MC-LR, during the first week of incubation might imply that the [D-Asp3]MC-LR pool is not stable and that the compound is synthesized, used and replenished constantly under the given culturing conditions. Growth, seems to be a conservative trait in *M. aeruginosa* because is preserved over toxin production which limits its application as ecotoxicological endpoint in risk assessment of pesticides. Microcystin production seems more responsive (sensitive) to glyphosate in time and at lower concentrations than growth.

ET13B-2

Physiological costs for biotransformation of cyanobacterial toxins in Dreissena polymorpha and Unio tumidus

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Freshwater mussels such as *D. polymorpha* and *U. tumidus* with high fitration may accumulate cyanobacteria and their toxins during cyanobacterial blooms. The population of Unionidae is endangered due to shoreline construction and water pollution as well as by competition for food with *D. polymorpha*. The two species are not competing for the same habitat, as the Unionidae submerges in soft sediment, whereas *D. polymorpha* attaches to hard substrat. In contrast to the native *U. tumidus*, the invader *D. polymorpha* establishes sustainable populations in urban watercourses. *D. polymorpha* seems to be a moderate sensitive species.
This study compares the freshwater mussels *D. polymorpha* and *U. tumidus* with regard to their physiological costs for the biotransformation of microcystin. For this, the energy reservoirs (glycogen- and lipid content) and the glutathione pool (total GSH, reduced GSH and GSSG) were compared in the invasive *D. polymorpha* and in the native *U. tumidus* in response to cyanotoxin exposure (10 and 50 µgL-1) for 24 h and 7 d. A further aim was to compare the effect of cyanotoxin exposure to fatty acid patterns in the two species.
Energy reserves and GSH pool of *D. polymorpha* were measured in whole mussel tissue and of *U. tumidus* in digestive gland, remaining tissue, gills, mantle and foot. Fatty acid composition were analysed in whole mussel tissue of both species. The glycogen content decreased after 24 h in both species indicating the energy requirements due to the stress caused by the MC-LR exposure. The lipid content was merely not affected in *D. polymorpha*, whereas the lipid content in remaining tissue, digestive gland and foot of *U. tumidus* significantly decreased. The decrease in the lipid content in *U. tumidus* indicates the additional consumption of the long-term energy reserves to deal with microcystin. The GSH pool was merely not affected by microcystin exposure in both species.
Exposure to MC-LR or cyanobacterial crude extract enhanced the requirement for energy, as indicated by reduced glycogen content in both mussel species and reduced lipid content in U. tumidus. Together with results from a further study on capacities of biotransformation of microcystin and the ability to cope with oxidative stress in D. polymorpha and U. tumidus, it seems that D. polymorpha is better adapted to cyanobacteria contaminated water courses in comparison to U. tumidus.

ET13B-3

Do cyanobacteria produce estrogenic compounds?

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Number of studies document hazardous potential of cyanobacterial metabolites. Despite the observed effects on reproduction related parameters, growth and development, there is little information on endocrine disruptive potential of compounds from cyanobacteria. Our research aimed to determine if complex water bloom of cyanobacteria collected from the environment contain compounds that would interact with the signaling pathways of arylhydrocarbon receptor (AhR), androgenic receptor (AR), estrogenic receptor (ER), glucocorticoid receptor (GR) or retinoid acid receptor (RAR). In vitro reporter gene trans-activation assays were used to determine the potency of samples to elicit receptor-mediated responses. Estrogenicity was detected for most environmental samples, their estimated estrogenic equivalents ranged from 19 to 2200 ng 17b-estradiol /g dw. Some of them also interfered with the signalling of androgen receptor. The follow-up study investigated in greater detail estrogenic potency of both inner content and exudates of individual cyanobacteria and algae cultured in laboratory. The results document estrogenic potential of compounds produced by cyanobacteria

and algae, generally more for the compounds excreted to their surroundings. Exudates from two algal species and from most tested pure strains of cyanobacteria elicited estrogenic activity with concentrations of estrogenic equivalents in the range of ng/L, which could be considered relatively large since equivalent potencies of estrogens have been shown to cause reproductive toxicity to aquatic animals. Aqueous extracts caused significant estrogenicity only in case of both algal species and one cyanobacteria (Aphanizomenon gracile) with estrogenic equivalents ranging from 15 to 280 ng 17b-estradiol/g DW. There does not seem to be any clear relation between the estrogenic potency and concentration of known cyanotoxins. The research was supported by the Czech Science Foundation grant No. P503/12/0553 and by the project CETOCOEN (CZ.1.05/2.1.00/01.0001) from the European Regional Development Fund.

ET13B-4
Seasonal variation of immune parameters in mussels (Elliptio complanata) exposed to natural bloom of cyanobacteria
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Toxic cyanobacteria can have harmful impacts on aquatic organisms. In Lakes located in the Eastern-township (Québec, Canada), *Microcystis aeruginosa* blooms drift into littoral areas. We studied the effects of *M. aeruginosa* on the immune system of a common mussel (*Elliptio complanata*) during an algal bloom. In order to verify the toxic potential of these algae, we immersed caged mussels during 4 weeks for three consecutive months, from August to October, in four different sites. Among these sites, three of them belong to the Yamaska Basin, which is contaminated by anthropogenic pollution (agricultural and industrial). Two of the sites (Boivin, Choiniere) are prone to cyanobacterial blooms during summer, a third site represented the Yamaska River outlet (Yamaska) into the St-Lawrence River and the fourth site was located in the Lac St-Pierre and is considered the cleanest site (LSP). *M. aeruginosa* bloom occurred in Choiniere in September. The immune parameters consisted in haemocyte integrity and phagocytosis were analyzed each month by flow cytometry. Two-way ANOVA (interaction term) indicated that immune parameters exhibited marked temporal variation in different sites. Haemocyte viability remained constant throughout the experiment in Boivin and Choiniere but a significant decrease was measured in September and in October in both Yamaska and LSP sites. At Choiniere, phagocytosis activity remained relatively constant after each monthly exposure even though a significant decrease was observed in both LSP and Yamaska in September. Mortality of haemocytes could be a sign of cytotoxicity and mechanical or chemical disturbance, which cyanobacteria bloom does not seem to be. Yamaska outlet incorporates all sources of pollution both agricultural and industrial but LSP results are quite surprising as it represents the cleanest site. A decrease of the phagocytic index is usually observed response to in vivo or in vitro exposures to toxic concentration of xenobiotics or a hormesis phenomenon. Consequently, ingestion of algal during a bloom by mussels does not provide evidence of a depressed immune status during *M. aeruginosa* bloom. Furthermore, the present study will investigate other effects of these toxins on the intracellular thiols production, reactive oxygen species (ROS) production, cyclooxygenase activity (COX) and nitrate production in mussel haemolymph.

ET13B-5
Impact of natural cyanobacterial biomass containing microcystin-LR on larval stages of the amphibian Xenopus laevis
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Cyanobacteria are known to produce several compounds and especially their secondary metabolites, so-called cyanotoxins, might have severe effects on vertebrates according to their chemical structures. The main occurring cyanotoxins, microcystins (MCs), are considered as being potent hepatotoxins causing a threat for health of vertebrates including humans. Despite aquatic vertebrates are primarily exposed to cyanobacteria and especially to cyanotoxins, in their natural habitat, most studies with MCs were performed using non-aquatic vertebrates e.g. mammals. In aquatic vertebrates most experiments have been carried out in fish by unnatural exposures via injections of pure cyanotoxins. In amphibians, only a few data are available to assess MCs impacts during embryonic development. However, only postembryonic larval stages are characterized as the most sensitive period concerning development and sexual differentiation. In the present study, tadpoles of the South African clawed frog Xenopus laevis were exposed for 21 days to MC-LR provided with diet as a natural exposure route. Effects of diets containing natural cyanobacterial biomass resulting in considerable amounts of 42.8 and 187.0 µg MC-LR/g diet, respectively, were investigated concerning bioaccumulation, development, stress and detoxification in order to determine for the first time potential physiological impacts of cyanobacteria on postembryonic stages of amphibians. The fate of MC-LR at concentrations of taken up via ingestion was determined in whole body using liquid chromatography-with tandem mass spectrometry detection. Beside mortality and weight effects on metamorphosis were assessed by recording developmental stages. In parallel, corticosteroid levels were determined as stress biomarker. In addition, gene expression of endocrine parameters associated with metamorphosis and sexual differentiation as well as of detoxification enzymes of all three biotransformation phases was investigated. Surprisingly, exposure to MC-LR containing cyanobacterial biomass applied via natural exposure neither resulted in bioaccumulation of MC-LR nor affected survival and development, only slight impacts on weight and corticosteroid levels were found. It seems likely that X. laevis underwent evolutionary adaptations to cope physiologically with toxic cyanobacteria occurring in the same habitat, enabling survival even at MC-LR concentrations being rather toxic for mammals.

ET14 - Bringing ecological processes into ecotoxicological risk assessment

ET14-1
Fungicidal effects on a decomposer-detritivore-system
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In heterotrophic streams, leaf litter breakdown is a fundamental process that is primarily realized by decomposing microorganisms, especially fungi, and detritivorous, leaf-shredding macroinvertebrates. Although fungicides, due to their unspecific modes of action, may affect both groups of organisms, up to date no test design exists to assess combined effects (i.e. direct toxicity and food quality related, indirect effects) on shredders. Therefore, the present study evaluated this scenario using a five-week semi-static test design with *Gammarus fossarum* as test organism and tebuconazole (65 µg/L) as model fungicide. Hence, gammarids directly exposed to the fungicide additionally received leaves conditioned (i.e. colonized and altered by microorganisms) in the presence of tebuconazole. Fungicide exposed gammarids produced significantly less faeces (~20%) resulting in a significantly increased assimilation (~30%). This increase probably compensated for direct, toxic as well as indirect (i.e. lower quality of food) effects. The latter were indicated by a significantly reduced fungal biomass (~40%) and a significantly reduced sporulation (~30%) associated with tebuconazole exposed leaves. However, the significantly reduced lipid content (~20%) of gammarids exposed to the fungicide implies that combined effects of tebuconazole were not fully compensated by the increase in assimilation. The present study, thus, indicates that fungicides may affect energy processing in gammarids, a key species in leaf litter breakdown. This may eventually translate into alterations in energy transfer in heterotrophic streams. Consequently, test designs similar to the one used during the present study - assessing combined effects - should be used in the future to validate the protectiveness of environmental risk assessment for decomposer-detritivore-systems and consequently leaf litter breakdown.

ET14-2
Trophic niche metrics based on stable isotopes (d13C and d15N) for the assessment of the functional effects of toxic substances on aquatic food webs
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Toxic substances may have negative impacts leading to cascading effects on food webs through e.g., changes in the nature and abundance of available food resources or inter-species relationships. This may lead to effects on various functional processes that ultimately translate into changes in ecosystem properties. The identification of parameters that may provide information on the effects on ecosystem functioning therefore constitute a challenge for the future of ecological risk assessment. Measurements of the natural abundances of stable isotopes of various elements in environmental samples are widely used in ecological studies to characterize the trophic position of species and to identify the sources and pathways of matter across food webs. Various quantitative metrics of trophic niche based on such analysis have recently been proposed, that proved to be efficient in identifying the effects of environmental degradation on aquatic food webs.

In this study the characteristics of the trophic niche of a freshwater snail species, Radix peregra, were characterized in outdoor control and fungicide-exposed artificial streams. Streams (length: 40 m, depth: 50 cm) were exposed to a dithiocarbamate fungicide, thiram. Two concentrations (nominal values: 35 and 170 µg L-1), were used, with two replicates per concentration. Four untreated cosms were used as controls. Streams were continuously exposed for three weeks, followed by a two month-long recovery period. Samples of snails and their potential food sources (filamentous algae, biofilm, litter) were collected in the streams just before the beginning and immediately after the end of the exposure period, and at the end of recovery period. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope signatures of samples were measured using mass spectrometry. The results were used to compute the values of various trophic niche metrics that were compared between control and contaminated systems. The results clearly show a significant effect of thiram on the characteristics of the trophic niche of R. peregra and on their dynamics. Changes in the structure of benthic food web in the streams were associated with changes in the nature and abundance of food sources used by R. peregra. Stable isotope signature of snail tissues mirrored these changes therefore suggesting that this approach may prove to be useful to assess some of the functional consequences of toxic substances on aquatic food webs.

ET14-3
The do's and don'ts of putting eco into ecotoxicology

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In 1988 John Cairns Jr. published a paper in Regulatory Toxicology and Pharmacology entitled 'Putting the eco into ecotoxicology'. In this paper he made a visionary plea for assessing effects of chemicals using mesocosms and to include this information in the ecological risk assessment process. The hope was to bridge the gap between the data gathered for the risk assessment (standard laboratory tests) and the protection goal (ecosystems). In the field of (chemical) stress ecology, ecological tools are used and adapted for inclusion in risk assessment. In this paper I will review the studies that uses species traits and the processes between species traits and the determinants of ecosystem response to chemical stress. The do's and don'ts will be identified and discussed as well as a way forward. Van den Brink (2008) defines vulnerability as a combination of intrinsic sensitivity, recovery potential and ecosystem interactions. Although sensitivity is not an ecological trait its inclusion in the ecological risk assessment of chemicals is pivotal. For the establishment of the relationships between traits and sensitivity or when sensitivity is used in retrospective risk assessment, mode of action specific approaches need to be developed. For a successful use of indices of chemical stress, their specificity should also be proven by assessing their correlation with other types of stress, like sedimentation, nutrient and habitat destruction. Recovery is determined by the sensitivity and life history characteristics, like dispersal ability and reproduction, of the species and the structure of the landscape under consideration. Therefore, population models are ideal tools to estimate of recovery for a particular species in a defined landscape. In order to improve the ecological foundation of risk assessments, there is a great need for modelling and experimental studies that addresses the importance of habitat quality, multiple stress and both intra- and interspecific interactions for the recovery of affected populations. Micro- or mesocosm experiments are considered a realistic and useful approach to directly assess chemical effects on ecosystem structure. Measurements of ecosystem functioning, however, are much more scarce. Food web models can either be used to assess the effects on ecosystem function from an existing mesocosm data set or can be used for the assessment of chemical risk for a certain ecosystem.

ET14-4
Predicting community effects of toxicants considering interaction with stressors
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Environmental risk assessment aims at predicting effects of PPP's on non-target communities so that unacceptable effects are avoided. To do so, a tiered approach is used that ranges from lower tier to higher tier testing, characterised by an increasing realism. Relevant processes to be considered in this context are interactions of toxicants with biotic and abiotic stressors. Such interactions can act in two directions: they may either reduce or enhance effects of toxicants. The identification of these effects is highly relevant to further improve risk assessment of toxicants. The aim of this contri-bution is to identify the possibilities and limitations of a systematic prediction of magnitude and direction of toxicant effects when interacting with biotic and abiotic stressors in aquatic ecosystems. We show that interactions of toxicants with biotic and abiotic stressors alter the sensitivity of populations. They can vary up to two orders of magnitude. Ecological traits that are relevant to predict outcomes of interactions were identified. Finally the results were related to the trait based indicator system SPEAR to validate predictions of toxicant interactions with stressors. We conclude that considering interactions with biotic and abiotic stressors greatly enhances the quality of the effect assessment of toxicants

ET14-5
Competition matters: modelling long-term population level effects of contamination
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Prospective risk assessment of pesticides aims at insuring that there are no adverse effects of the substances on the environment. Recently, experimental studies revealed the high relevance of species competition for toxicant effects on the population and community level. Hence, for risk assessment to be realistic and to include all relevant ecological processes, competition should be taken into account.
Here, we use a generic individual-based model (IBM) of two competing species to investigate how acute and chronic toxicant effects on individuals translate to population level effects. In addition, we investigate the role of life cycle traits on population recovery. Furthermore, we compare our modelling results with data of population dynamics observed in a semi-field study.
We found that competition substantially prolongs population recovery after contamination: Without interspecific competition the population of the sensitive species recovered within 9 time steps after a contamination event that killed 50% of the individuals. In contrast to that, recovery time increased to up to 34 time steps in the presence of an insensitive competitor. Furthermore, chronic toxic effects on the individuals that decreased reproduction capacity or survival probability increased the adverse effects on the population level. In particular, species with lower reproduction capacity were strongly affected in the presence of competitors and additional chronic effects. In extreme cases populations of such species eventually went extinct even with low acute and chronic effects of the toxicant on the individuals. We showed the structural realism of the model as it produced comparable pattern of the relationship between the tow competing species abundances as observed in the semi-field study.
Our findings imply that both experimental and modelling studies that do not consider the interaction between interspecific competition and toxicant effects will largely overestimate the speed of recovery processes. The inclusion of species interaction in ecotoxicological models aids a more realistic prediction of long-term effects on the population level from effects on the individual level.

ET14-6
Using a modelling approach to compare sensitivities to Triphenyltin at the individual and population levels for three planktonic organisms
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Extrapolation of toxic effects from the individual to population level requires the consideration of detailed underlying processes. Species differ in life-history strategies and consequently in their responses to toxic stress, population dynamics and time to recovery.
In this study an individual-based population modelling (IBM) approach for the freshwater cyclopoid copepod *Mesocyclops leuckarti*, the multivoltine aquatic phantom midge *Chaoborus crystallinus* and the cladoceran *Daphnia magna*, was used to simulate the responses of populations to Triphenyltin (TPT). Acute toxicity data for *M. leuckarti* with TPT (LC_{50} between 50 and 60 µg/l for copepodites and adults), *C. crystallinus* (LC_{50} 19 µg/l) and *D. magna* (LC_{50} 30 µg/l for neonates, 50 µg/l for adults) for constant exposure to TPT came from laboratory experiments. We used a newly developed IBM for *M. leuckarti* and existing IBMs for *C. crystallinus* and *D. magna*. The IBMs for each of these species are based on their respective physiological parameters and life-history. Each IBM is distinct in terms of the life cycle of the species it describes. The General Unified Threshold model of Survival (GUTS) was employed to simulate the toxicokinetics (TK) and toxicodynamics (TD) of TPT. The endpoints evaluated were extinction probability for constant exposure and time to recovery for a single peak application.
For similar acute sensitivities in laboratory experiments, which is also what we observed, microcosm experiments by Roessink (2008) showed different responses at the population level e.g. NOEC values for chaoborids, cladocerans and copepods in clean sediments were 10 µg/l (week 8 to 42), 30 µg/l (week 0.4 to 12) and 1 µg/l (week 2 to 8) respectively. For a single application of TPT, cladocerans and copepods recovered in 4 weeks and 12 weeks post application at 30 µg/l whereas chaoborids did not recover at all. We used our IBMs to simulate these data sets and to try to predict the observed varied sensitivities and recovery times.
In conclusion, for species with similar individual level responses to toxicants, population level responses may be vastly different due the influence of different life-history strategies. It is possible to simulate these differences using the power of population modelling and make educated predictions for better risk assessment.

ET15 - Pesticide fate and ecotoxicology

ET15A-1
Implications of the inclusion of acute tests with Americamysis bahia and/or Chironomus riparius as new data requirements for the aquatic effect assessment of insecticide
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Under the new PPP regulation the acute toxicity to aquatic invertebrates will, in first instance, be assessed on basis of (1) the acute lab 48-h EC50 for Daphnia and (2) the acute 48-h EC50 for Americamysis bahia and/or Chironomus riparius. In the first-tier effect assessment an Assessment Factor (AF) of 100 will be applied to the lowest 48-h EC50 value. The tier-1 Regulatory Acceptable Concentration (tier-1 RAC) thus obtained should not exceed the highest predicted peak concentration (PECmax) in edge-of-field surface water. We intend to evaluate the implication of the new aquatic data requirements (inclusion of acute tests with A. bahia and/or Chironomus spp.) for the tier-1 acute effect assessment for insecticides by using threshold concentrations for treatment-related effects as observed in micro/mesocosm studies treated with insecticides.
Our evaluation demonstrates that for insecticides with very specific modes-of-action, such as neonicotinoids and insect growth regulators (IGRs), the concentration on basis of the EC50 of Daphnia and the application of an AF of 100 may not always protect sensitive arthropods in micro/mesocosm tests. The tier-1 effect assessment procedure on basis of the new data requirements (acute EC50 values for Daphnia magna and Americamysis bahia and/or Chironomus) appears to be protective for the vast majority of insecticides evaluated in micro/mesocosms. For the combination D. magna and A. bahia only in 2 out of 21 cases the tier-1 RAC was not protective, while that was 1 out of 25 cases for the combination D. magna and Chironomus and 1 out of 29 cases for the combination D. magna and either A. bahia or Chironomus. Overall, the current evaluation

by comparison of RACs obtained from the new first-tier data requirements to ecological safe threshold concentrations for arthropods communities as derived from semi-field tests implies that the new data requirements in case of insecticides and using acute toxicity data from D. magna and A. bahia or an OECD-chironomid seems to be protective in most cases and is an improvement compared to the old data requirements.

ET15A-2
A focal fish species for pesticide risk assessment in the EU
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Extrapolation is needed to link standard toxicity endpoints to protection goals. Ecological modelling can serve as an extrapolation tool from the individual to the population level and from test to exposed species. However, it is crucial to decide on a suitable species to be modelled. For this purpose, we established a procedure to identify a focal species, a realistic-worst case which is thus representative of the life histories that might occur in the field, on conservative basis. We adopted the vulnerability principle which consists of (a) intrinsic sensitivity, (b) external exposure and (c) ability to sustain a population. The procedure allows the definition of a focal species assuming that all fish can be intrinsically sensitive to certain levels of plant protection products (PPPs).
Out of 579 fish species living in European freshwaters, 27 were found to be native to Europe, widespread in at least one of the EU regulatory zones of authorization of PPPs, and inhabiting streams, ditches or ponds. Based on the reviewed studies on species assemblages, the presence of these species in agricultural settings was verified, and hence their potential external exposure to PPPs.
Deterministic age-based matrix models (time step 1 year) are being parameterised from published field demographic studies. The procedure was exemplified so far for four species whereby proportional effects of alterations in juvenile survival, adult survival or fertility on population multiplication rates (λ) were compared. Since fish are protected on the individual level against visible mortality, the most important factor in our analysis is the reduction in fertility and its effects on the population multiplication rate of a species.
The focal species will be identified and presented; a fish species that is native to Europe, widespread in the EU, lives in edge-of-field water bodies and has the lowest ability to sustain its population (highest proportional effect or elasticity of λ) as compared with the other species in case a PPP reduces its fertility and thus compromises this ability.

ET15A-3
In situ GamTox for assessment of acute and chronic pollution in small streams
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ET15A-4
Sub-lethal effects of imidacloprid pulses to aquatic invertebrates
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Observing behavioural changes of organisms evoked by relatively short and low concentrated stress in ex-situ studies is a useful tool to address xenobiotic impacts in a more environmentally-relevant manner. Ecosystems are usually exposed to low concentrations of toxicants and environmentally-relevant concentrations rarely cause mortality nor are continuously present.
We aimed to contribute to the understanding of the time course of sub-lethal effects of short-term exposure with xenobiotics to non-target species by undertaking ex situ studies with a recovery phase. The feeding rate of the shredder Gammarus pulex and a range of sub-lethal endpoints for the crustacean Daphnia magna were observed. We measured the influence of the neonicotinoid insecticide imidacloprid to the feeding rate of G. pulex within a feeding activity assay consisting of a four day exposure phase extended with a five day recovery phase. The impact of a one-week exposure of new born D. magna to imidacloprid was observed measuring growth and reproduction subsequent to exposure frequently over four weeks.

We found that concentrations within the range of measured and estimated environmental concentrations caused significant decreases in the feeding activity of G. pulex during exposure, and that a strong rebound in feeding activity occurred after exposure. Effects of imidacloprid to the growth and reproduction of D. magna were observed within and after exposure. Both endpoints were still concentration dependent and significantly affected four weeks after exposure. Inhibition in growth was observed at the lowest concentration tested (0.15 mg/L) whereas inhibition in reproduction was observed at concentrations of at least one order of magnitude higher.
Lethal and sub-lethal endpoints can be still valid beyond the presence of the toxicant. Observing the endpoint of interest above the direct impact can lead to a better understanding of the effect. Whether or not sub-lethal effects of toxicants at an individual level are ecologically relevant needs investigation. Ecotoxicological modelling, in particular individual-based population modelling, is a rising research area trying to overcome the mismatch between laboratory testing on individuals and the aim of environmental risk assessment to protect populations. Data as shown here not only contribute to the understanding of effects, but they can also be used for development and evaluation of models.

ET15A-5
New methods for assessing the effects of insecticides on insect larvae and adult emergence in freshwater outdoor microcosms
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In the classic test designs that follow current recommendations for freshwater microcosm studies, (HARAP, CLASSIC, and OECD), the effects of insecticides on the emergence of insects from aquatic systems are generally based on counts of their numbers in emergence traps that occupy a relatively small proportion of the water surface. In order allow a more criticial examination of insecticide effects, in this presentation we describe the application of completely new methods for assessing effects on larvae and on adult emergence, as well as effects on the reproduction of some species. Field microcosms were covered by enclosures to form a walk-in aerial microcosm (24 m3) which retained emerging insects for collection and also provided the opportunity for insects in the enclosure to reproduce and re-introduce larvae back into the aquatic microcosm.
The results of two studies on insecticides with differing physicochemical properties are described. In the first, over 155,000 insects from 13 families were collected. Emergence times for some species e.g. Damselflies (Coenagrionidae) and Mayflies (Baetidae) were clearly defined whereas others e.g. Caddis (Lymnephilidae) and the Phantom midges (Chaoboridae) were protracted and spanned almost the entire experimental period. Insect numbers were sufficient for multivariate and univariate analysis but differences in emergence periods highlighted the need for a knowledge of life history to allow correct data interpretation. In the second study, where emerged insects were allowed to survive within the enclosures, larval counts in invertebrate colonisers placed into microcosms showed that at least three insect taxa i.e. Lymnephilidae (Caddis), Coenagrionidae (Damselfly) and Lestidae (Damselfly), reproduced and deposited eggs back into the microcosms from which they themselves emerged.
The methods described here can be used to assess the effects of plant protection products on insect emergence and reproduction under realistic semi-field conditions which satisfies a key requirement for the determination of the environmental safety of insecticides. The insects identified in these studies that were capable of emerging and reproducing in the aerial microcosms formed by the field enclosures, provide a valuable resource for extending field methods in ecotoxicology.

ET15A-6
Low concentrations of imidacloprid cause mortality of Gammarus pulex by interfering with feeding behavior
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Neonicotinoids are insecticides which interfere with invertebrate behavior, e.g. feeding, by mimicking neurotransmitter acetylcholine and stimulating the nervous system. If feeding behavior is impaired, neonicotinoids might cause mortality even in concentrations far below acute toxicity levels. We studied effects of the neonicotinoid imidacloprid on the aquatic invertebrate *Gammarus pulex*. *G.pulex* is an European amphipod decomposing organic material in streams. It is highly affected by imidacloprid: acute toxicity to *G.pulex* (LC50 = 270 µg/L) is almost 800 times below the concentrations for fish.
We carried out two experiments (14d and 21d long), where we measured survival, feeding rate and lipid content. Both included two treatments (A, B) with two 1-d exposure pulses and one treatment with constant exposure to the corresponding time-weighted average concentration (C: 15 µg/L). Treatments A and B differed in their times between the chemical pulses (A: short, B: long). In the 1st experiment, we observed only survival while during the 2nd experiment, we also observed feeding rate by weighing the food before providing it to *G.pulex* and after removal from experimental beakers. Lipid content was measured from organisms sampled at the end of the 2nd experiment, by the gravimetric method. For food we provided horse chest-nut leaves, inoculated with the fungi *Cladosporium herbarum*.
At the end of the 1st experiment, survival in treatment A was 70%, in treatment B 63%, in treatment C (TWA) 46% and in controls 82%. The difference in survival curves between treatment C and controls was highly significant (one-way ANOVA: $p = 0.0001$) and also the pulse treatments differed from controls (A: $p = 0.091$, B: $p = 0.014$). Feeding rate measured during the 2nd experiment did not differ significantly between controls and pulsed treatments (Repeated Measures ANOVA: $p > 0.05$) while between

treatment C (TWA) and controls, the difference was highly significant ($p < 0.001$, C: 1.94 ± 0.85 mg/individual/day, controls: 4.42 ± 1.82 mg/individual/day). In addition, lipid content at the end of the 2nd experiment differed between treatment C and controls (t-test: $p = 0.027$, C: 1.1 ± 0.7%, controls: 1.5 ± 0.6%). The difference between survival curves of treatment C and controls was not significant ($p = 0.076$).
Altogether, the results indicate that low concentrations of imidacloprid inhibit feeding of *G.pulex*, which may lead to mortality due to combined effect of the compound and starvation.

ET15B-1
Using 'species sensitivity distribution' to assess herbicides toxicity on benthic diatoms assemblages
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Herbicides in the littoral zone of lakes are often present in higher concentrations with greater molecule diversity than in pelagic zone. Benthic diatoms comprise a significant portion of the fixed biomass in this zone, but the herbicides tolerance threshold of these non targeted organisms is not well known. With the view of assessing the ecological risk of herbicides for aquatic microorganisms, some models, such as Species Sensitivity Distribution (SSD), have been developed on phytoplanktonic species of the pelagic zone. Often, benthic communities are not considered in lake from an ecotoxicological point of view. However, these zones could be exposed to specific contamination and studying the sensitivity of benthic organisms could provide a new approach for ecotoxicity assessment in lacustrine environments. Our study aims to determine if SSD models are adapted to describe the sensitivity of benthic diatoms to herbicides. To achieve this goal, we assessed the sensitivity of 11 benthic diatoms species to 5 herbicides (diuron, isoproturon, terbutryn, atrazin and metolachlor).
First, we constructed a database of toxicity thresholds for each herbicide-species combination using 96 hours monospecific bioassays and an evaluation of growth inhibition. From each dose-response curve, an effective concentration that inhibits 50% of growth (EC50) was extrapolated. EC50 values showed a great variability of sensitivity among species for a same herbicide and among herbicides for the same species. A SSD curve (SSD-EC50) was built for each herbicide, based on EC50 values of each species exposed to that herbicide. According to Hazardous Concentrations (HC) obtained from SSD-EC50 curves, the increasing order of herbicide toxicity was metolachlor, atrazin, isoproturon, terbutryn and diuron. Different pools of diatoms were defined according to their tolerance level. The most obvious pools distinction was associated with photosystem II (PSII) inhibitors. Four strains (*Nitzschia palea*, *Craticula accomoda*, *Gomphonema parvulum*, *Eolimna minima*) of the 11 tested species were always the more resistant. This tolerance could be explained by the trophic mode and the motile guild. Indeed, N-heterotroph and motile guild species seemed to be more resistant to PSII inhibitors than N-autotroph and other profile guild species.
These initial results are encouraging and support the use of SSD models for more complex investigations based on benthic diatoms data.

ET15B-2
Ecotoxicity testing of pesticides at the soil surface using a non-target terrestrial gastropod
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Fate and effects of pesticides in agrosystem were studied using a non target species living at the interface soil-plant-air, the landsnail Helix aspersa. Fate in and effects on snails of 8 commercial herbicide and fungicide formulations (Roundup® and Bypass® = glyphosate; Basta® = glufosinate; Valiant® Flash = folpet + cymoxanil + fosetyl-Al; Corail® = tebuconazole; Cabrio® Top = pyraclostrobin + metiram-Zn; Thiovit® = sulphur; Bordeaux mixture = copper salts) commonly employed in vineyards were studied in the field and for some of them, in laboratory experiments. Here we present a synthesis of a four-years program that aims to develop various biological methods for in situ biomonitoring and life cycle exposure under controlled conditions.
A field study in real conditions of pesticide application revealed few effects on the survival and growth of juvenile snails exposed in microcosms to each different treatment. Glyphosate (4 mg kg-1) and AMPA (8 mg kg-1) were detected in snail tissues. The transfer of pyraclostrobin and tebuconazole was also demonstrated. Internal concentrations were low but however higher than LMR drawn for animal products.
The effects of two herbicides, Bypass® and Basta® were studied on the whole life cycle of snails in laboratory conditions. No toxicity was found on survival and growth although an accumulation of glyphosate was revealed in snails (6 mg kg-1) exposed continuously to contaminated food (30 mg kg-1).
Bioassays for embryotoxicity testing of chemicals in solution or in the soil showed that all pesticides inhibited egg hatching success at lower concentrations than those applied in the field and the influence of soil characteristics on the toxicity of the pesticides was demonstrated. As bioassays using eggs are considered as in vitro methods, efforts should be given to improve and promote the use of such method.
This work led to the proposal of complementary tools available for pesticide risk assessment in terrestrial environment. Analytical difficulties were encountered and it was not possible to finalize the analytical methods for all substances studied. Thus, the determination of pesticide residues in animal tissues still represents an essential challenge for current environmental concerns.

ET15B-3
Dust drift during sowing into adjacent areas - potential emission during sowing of seeds treated with pesticides
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Seeds of many crops are pesticide treated to protect young seedlings against pests. In 2008 bee poisonings of about 12000 hives was observed in Germany after sowing of maize. Relevant amounts of insecticides drifted into adjacent flowering crops in the form of contaminated dust. Analysis of dust abrasion from different seed batches resulted in up to several g a.s. / ha in the form of loose dust.
In field experiments using seed batches with known content of loose dust and a.s. in dust different passive samplers (petridishes, adjacent crops, gauze nets) were used to check for potential exposure in the neighbourhood of sown fields. Data of field experiments carried out between 2008 and 2011 will be presented which show potential exposure due to sowing of different crops and seed qualities. Differences in spray and dust drift measurement will be presented.
Residue analysis in adjacent crops compared to values in petri dishes on uncovered soil at the same distance of sowing machinery indicate between about 1 - 5 times higher amounts of residues in crops calculated per m² than in petri dishes on bare soil. Adjacent crops seem to filter dust, so higher rates per m² were detected near to the sowing area in plants compared to petri dishes. Residues in vertical gauze nets were about 3 - 10 times higher compared to petridishes in the same distance of sowing.
Therefore drift residue data of petridishes should only be used carefully to predict exposure for non target organisms such as honey bees in adjacent 3D structures such as plants.

ET15B-4
How important are pesticides for the ecological integrity of large rivers and what are their effect thresholds in freshwater ecosystems?
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Several field studies in small agricultural streams ranging from a local to regional scale have demonstrated that pesticides may affect freshwater ecosystems. However, it is unclear whether effects are limited to small streams in agricultural areas, whether pesticides are more important than other chemicals and what are the effects thresholds in the field. In order to answer these questions we compiled two data sets. First, the detection frequencies and concentrations for 331 organic compounds measured in the four largest rivers of North Germany over a period of ten years were analysed including an assessment of the ecotoxicological risk to standard test organisms. Second, we compiled data from nine regional field studies from Europe, Siberia and Australia in order to derive thresholds for the effects of pesticides on macroinvertebrate communities and the ecosystem function leaf breakdown. For the rivers in the first data set, the detection frequency for most organic compounds decreased significantly over the ten year study period and polycyclic aromatic hydrocarbons (PAHs) were most frequently detected. Nevertheless, pesticides were the most important chemical group concerning toxicity for the standard test organisms and occurred in concentrations envisaging acute toxic effects. Regarding effect thresholds, dose-response models for the relationship of pesticide toxicity with the abundance of sensitive macroinvertebrate taxa showed significant differences to reference sites at levels of 1/1,000 to 1/10,000 of the median acute effect concentration (EC50) for Daphnia magna. Hence, the analysis revealed effects well below the threshold of 1/100 of the EC50 for D. magna derived from mesocosm studies that corresponds to the threshold incorporated in the European Union Uniform Principles for pesticide registration. The invertebrate leaf breakdown rate was linearly related to the abundance of sensitive species in the communities and decreased in concert with a decrease in the sensitive macroinvertebrates, though only for two of the three countries for which data was available. Our results suggest that pesticides should be taken into account as a relevant stressor in river basin management and that the current thresholds used in the risk assessment framework are not protective for field communities.

ET15B-5
Pesticides effects on soil and adjacent water systems under Mediterranean crop-based exposure scenarios using a semi-field methodology
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Exposure and effects of pesticides on target and non-target organism may differ according to the natural variability of the ecosystem due to differences in climate and soil characteristics among others. Therefore, the need to study pesticide effects under Mediterranean conditions is of critical importance due to the lack of information on pesticide behavior and its effects on terrestrial biota for this region. In order to advance the knowledge on pesticide behaviour in soils and its routes of entry into adjacent aquatic systems as well as to study their lethal and sublethal effects on biota a semi-field experiment (soil-water simulator) was undertaken. The experimental design was based on simulations of realistic "worst case scenario"applications of recommended doses of three pesticides (azoxystrobin, chlorothalonil, ethoprophos) on highly irrigated crops (onion, potato, maize) using natural soil under Mediterranean climate (temperature and rain-event). Soil and water samples were analyzed for pesticide residues and tested for acute and chronic toxicity with Folsomia. candida, Eisenia andrei and Daphnia magna. Azoxystrobin moved to the water compartment, mainly through leaching most likely due to the high irrigation. As a result, toxic sublethal effects due to leachate and lethal effects due to runoff samples were observed towards D. magna, at the applied dosages. Runoff waters from rain events 10 days after chlorothalonil application can cause effects on the aquatic microcrustacean. Both scenarios do not threat non-target invertebrate soil biota under the applied dosages. Ethoprophos stayed practically immobile in the soil nevertheless pesticide residues detected on leachates and runoff samples were sufficient to cause lethal effects on the cladoceran. A high impact on both terrestrial species survival and reproduction was observed. The studies proved that water compartment close to agricultural fields and terrestrial communities are affected when highly irrigation is present and natural soil characteristics influence pesticides properties.

ET15B-6

Consequences of the Three Gorges Dam in China - Conceptual approaches to study the fate, bioaccumulation and effects of organic micropollutants in aquatic food webs and sediments of the Yangtze

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The construction of the Three Gorges Reservoir caused the flooding of former urban, industrial and agriculturally used areas. Consequently, substantial amounts of organic and inorganic pollutants are continuously released into the reservoir. Beyond further contaminants and nutrients (e.g. nitrate, phosphate) enter the reservoir by runoff from adjacent agricultural areas as well as from sewage of industry, aquacultures and households. Periodical changes in water level cause flooding events and thereby a relocation of contaminated water, particulate matter and sediment onto agriculturally used areas along the reservoirs shore. The main aim of the presented project is to identify major processes that determine the fate and degradation of organic contaminants, their potential bioaccumulation and the ecotoxicological effects of polluted sediments on aquatic organisms within the TGR. We focus on the specific alterations of the behavior and the effects of organic pollutants that are caused by the unique conditions within the new reservoir body [1]. We present our conceptual approach including the definition of contamination scenarios. First fate studies of 14-C radiolabelled model pesticides in water-sediment systems point to potential bioaccumulative metabolites. The simulation model AQUATOX was modified and used for preliminary modeling experiments which revealed the particular importance of the nutrient regime on the composition of the aquatic food webs and its implications for the bioaccumulation. Sediment samples of the Kaixian region have been tested in the Fish Embryo Toxicity Test and the Sediment contact Assay. These findings already serve as a starting point for a subsequent project on management strategies to reduce the pollution in Yangtze flooding areas.
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ET16 - Quantitative Structure Activity Relationship (QSARs) and similar models for predicting the toxicity of chemicals, mixtures and combined stress

ET16-1

VEGA, a new platform combining QSAR and read across for the prediction of chemical properties

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Under European regulations, including REACH, QSAR and read across are identified as acceptable methods for obtaining valid estimations of the properties of chemicals. The *VEGA platform* (*Virtual models for Evaluating the properties of chemicals within a Global Architecture*) offers free access to specialist software designed for QSAR predictions and read-across. VEGA is freely available online: *http://www.vega-qsar.eu*.
There are several QSAR models within VEGA, taken from CAESAR and T.E.S.T. software. VEGA can be used to predict BCF, fish and daphnia toxicity, logP, mutagenicity, carcinogenicity, developmental toxicity and skin sensitization. The models can be used on line, or you can download them.
VEGA introduced an applicability domain index (ADI) to evaluate if the result of the QSAR models is reliable. The ADI includes an assessment of the experimental values of the most similar compounds, and of the predictions of these compounds. Thus, the ADI goes beyond the typical approaches, which are based on chemometric measurements. However, even if the evaluation of the QSAR model through the ADI may identify critical issues, the user should evaluate the results obtained for the similar compounds, and then decide if the prediction is reliable. We verified that the predictions for the chemicals with higher ADI values have on average lower prediction errors. A stricter ADI redices the prediction coverage, of course.
VEGA implemented a series of tools showing the role of relevant descriptors, or the occurrence of important fragments. For each model you can see the role of descriptors and fragments for the chemical of interest and the similar compounds, .
VEGA shows the uncertainty associated with the prediction, depending on the different ADI. On the basis of these errors, in order to provide a conservative classification, we added a safety value to the predicted one. Thus, the prediction uncertainty is used to set the confidence innterval for the prediction.
VEGA has been checked for robustness by testing it on 4 million unique chemical structures.
Acknowledgement
We acknowledge financial support from the European Commission, Projects ORCHESTRA and ANTARES, and from the Ministero della Salute, Italy. The software T.E.S.T. has been developed with the financial support of the US EPA (Cincinnati, OH). The software CAESAR has been developed with the financial support of the European Commission, Project CAESAR.

ET16-2

Intracellular localization of chemicals

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Intracellular localization can to a large part be predicted from common physico-chemical properties, such as log Kow, pKa, valency and molar mass. The selective distribution of molecules in intracellular compartments such as membranes, mitochondria and lysosomes will also lead to specific diseases. Consequently, intracellular distribution may be a determinant of specific toxic effects of molecules. The cell model was used to predict the chemical space of compounds that localize in cellular subcompartments, such as cytosol, acidic vesicles (vacuoles or lysosomes), nucleus, mitochondria and oleosomes. Accordingly, weak acids localize in cytosol and stronger in mitochondria. Weak mono- and bivalent bases accumulate in acidic vesicles such as lysosomes and vacuoles. Strong bases (i.e., cations) and weak bases that form cations with delocalized charge are attracted by the electrical potential of mitochondria. Lipophilic molecules (log Kow > 5) partition of course primarily into membranes and lipids. Similar predictions are obtained using the Horobin QSAR model. A validation study for mitochondriotropics gave about 2/3rd agreement. Disagreement could be explained in most cases. The intracellular localization is not always selectively, making literature references sometimes ambiguous. A recent experimental study gave good agreement of cell model predictions with the experimentally determined dynamics of uptake. In conclusion, the prediction of intracellular localization from molecule properties by QSAR and pharmacodynamic models is feasible and eventually may be exploited to identify new, specific modes of action.
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ET16-3

Developing read-across justifications in REACH
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Under REACH, 'alternative methods' such as Read-Across and Grouping/Category are key tools for preventing unnecessary animal testing and reducing costs for dossier preparation. However, relatively little guidance on the identification of valid analogues and categories and whether categories will support only 'interpolation' or also 'extrapolation' is available. In this poster, we will present and develop practical examples of what we think are valid argumentation for these approaches. Read-across Read-across implies that measured data for one substance are used as surrogate data for another closely related substance, under the assumption that the chemical similarity of the two substances will result in biological similarity. Read-across can range in scope from a single end point to an entire dataset, and may involve using available source end point data to substitute for unavailable target data or to argue that tests need only be done for the designated source. A testing proposal submitted by a client for 2 closely related alkylphenols argued that the test was only needed for one substance, with subsequent read-across to the other; prompted by a rejection from ECHA, we developed more thorough argumentations for read-across based on available toxicological data and an in-depth description of the chemistry of both substances. Category Category approach implies that classes of closely related chemicals (usually homologous series) exist for which it is possible to qualitatively interpolate properties for 'missing' substances (analogously to the quantitative argument for classical QSARs). For a long chain alcohol, we prepared an argumentation that category approaches may also be used for near-end extrapolation and category extension. 'Mixtures' Data availability is often a problem for UVCB substances; moreover, their variable nature presents signficant challenges for testing and interpretation of results. Treating complex substances as mixtures allows the use of available data for individual constituents, if these are known, in an exercise akin to read-across, but prescribed methods often lead to over-prediction of hazard and classification. ENVIRON used a mixture approach to develop an integrated data set for a well-defined reaction product. By careful evaluation of the data set, supported by limited testing and refining evidence for degradation and bioconcentration, we were able to justify more reasonable PNECs and avoid classification of the substance.

ET16-4
Immobilised artificial membrane (IAM) chromatography : an investigation of the effect of pH on ionisable species
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The octanol/ water partition coefficient (log P) is used in many areas of ecotoxicology and modelling as a measure of hydrophobicity or lipophilicity. However it is well known that for ionisable chemicals or surfactants the measurement of log P is difficult due to the tendency of these types of materials to sit on the octanol/water barrier. Similarly, the accuracy of the prediction of log P for these types of materials is variable depending on the model used to calculate it. However, even accurate determination of log P does not always lead to accurate in silico correlation with toxicological endpoints. In part this is due to differences between octanol/water partitioning and partitioning across biological membranes. Phosphatidylcholine (PC) is a ubiquitous phospholipid which is a major component of biological membranes. Immobilised Artificial Membrane (IAM) Chromatography utilises a phospholipid membrane made of PC. Using PC as a stationary phase and aqueous or organically modified buffers as a mobile phase, a partition coefficient (k¬IAM) for chemicals can be determined as a measure of the ability of a chemical to partitiion into the liposome layer (phospholipophilicity).This technique has been shown to have good correlation with ecotoxicological endpoints for neutral compounds where log k¬IAM is measured. As in biological systems and in buffers, ionisable compounds exist in different ionic states which means that they interact to a different extent with biological membranes. The methods used to determine log P in these systems do not sufficiently allow for this which is why in part that correlation of ionisable chemicals with ecotoxicological endpoints becomes reduced. This study was designed to determine the effect of the pH of the system on the retention of the chemicals in their ionic and neutral forms using IAM chromatography. As expectected, the data indicated that the retention of ionisable chemicals is affected by the pH. Chemicals had a value for the neutral form (log kIAM) and the ionised form,(log kIAM (ion)) under relevant extreme pH conditions. However, at pH between these extremes, retention of the chemicals were based on an aggregate of both neutral and ionised species. It was also recorded that this aggregate retention indicated that the extent of ionisation of the system was not directly linked to the pKa of the compound under aqueous conditions but was shifted positively along the pH scale for acids and negatively for bases.

ET16-5
Simulation of metabolism and modelling environmental fate and (eco)toxicity of chemicals
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Information regarding the metabolism of xenobiotic chemicals plays a central role in regulatory risk assessments. Because so many new chemicals are being produced than can be assessed for potential hazards, setting assessment priorities among the thousands of untested chemicals requires methods for predictive hazard identification. Metabolites and breakdown products may occur in every environmental compartment, in animal and human food causing unexpected changes of evolutionary established biological communities or chronic adverse effects. For this reason data on the extent of formation of metabolites is required by regulatory authorities as specified in technical guidance documents. The aim of this presentation is to share our experience in the computerized management of metabolic data and development of simulators of metabolism for predicting environmental fate and (eco)toxicity of chemicals. Mathematical formalism and its adaptation for modeling environmental fate (abiotic and microbial degradation, bioaccumulation) and (eco)toxicity of chemicals (acute aquatic toxicity, skin sensitization, genetic toxicity) will be presented. Model performance, applicability domain and interpretation of model predictions and supplementary information will be also discussed.

ET16-6
Integrated screening classifier for substance-specific waiving of biodegradation assays in PBT assessments
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The implementation of the REACH regulatory framework requires the assessment of persistent, bioaccumulative and toxic (PBT) potential for substances that are produced or imported in amounts of 10 or more tones per year. The PBT assessment entails detailed substance-specific testing, which involves substancial use of animals and significant economic costs. In this context, the use of intelligent testing strategies (ITS) emerges as a cost-effective assessment scheme. A key element for the success of the ITS is the use of non-testing methods as complementary sources of evidence to waive specific bioassays. Therefore, the use of ITS to optimize the costs of regulatory risk assessment requires the development of highly reliable non-testing methods suitable for test waiving.
Persistence is a fundamental property that strongly determines the potential environmental impact of a chemical. Consequently, the objective of the current work was to develop and validate an integrated screening classifier specifically designed to identify non-ready biodegradable chemicals with high reliability (i.e. low risk of false negatives). Results obtained demonstrated that the use of ensembles of weak classifiers, tuned to distinguish between ready and non-ready biodegradable chemicals, constitute an appropriate approach to develop screening models suitable to identify non-ready biodegradable chemicals with high accuracy (99.7% and 100% respectively). The low false negative rate of the above ensemble classifiers allows its integration within the decision-making process of an ITS to waive biodegradation testing for certain chemicals.
The bioassay waiving that may result from the use of this approach will impact PBT assessments in terms animal use and economic resources needed, thus providing a cost-effective implementation of the REACH regulations.

ET18 - Tropical ecotoxicology

ET18-1
Assessing the toxicity of herbicides mixtures used in sugarcane culture in Brazil using zebrafish
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Brazil is a pioneer in large-scale use of ethanol, a renewable fuel that contributes to the reduction of greenhouse gas emissions by combustion of fossil fuels. The high productivity of sugarcane crops demands the intensive use of equipment and inputs that can contaminate water bodies adjacent. Herbicides are the most common pesticide used in this crop, but the impact of this practice on non-target organisms is not well known. Moreover, possible interactions between different herbicides used simultaneously are not understood. Most studies into herbicides ecotoxicology are related to temperate ecosystems thus, there are still many gaps about its effects in a tropical climate. The aim of this work was to evaluate the effects of the mixture of ametryn (AMT) and diuron (DIU), herbicides widely used in sugarcane crop, to the tropical zebrafish (Danio rerio). Binary combinations of DIU and AMT were tested as follows: 0, 4.8, 6.8, 9.6, 13.6, 19.2 mg L-1 of DIU and 0+ (solvent control); and 0, 0+, 9.7, 13.7, 19.4, 27.4 and 38.8 mg L-1 of AMT. The trials were based on the OECD guideline on Fish Embryo Toxicity Test. Several sub-lethal and lethal endpoints were evaluated. The activity of the biomarkers CHE, LDH and GST was determined after 96 h of embryos exposure to the binary mixture: 0, 0+, 2.5, 3.5, 5.0, 7.1 and 10.01 mgL-1 DIU; and 0, 0+, 5.1, 7.2, 10.1, 14.2 and 20.0 mgL-1 of AMT. Sigma Plot 10.0 package was used for statistical analyses and ToxCalc spreadsheet were used to calculate LC50-96h and determine the model that best describes the mixture. LC50-96h values for zebrafish exposed to AMT and DIU were, respectively, 48.46 (2.2) mgL-1 and 23.93 (1.24) mgL-1. The model which best describes the mixture was the independent action, with toxicity dose level-dependent: synergism in low and antagonism in high dose. GST activity was induced by AMT. However, when the mixture had higher concentrations of DIU we observed a reduction in activity. We observed an inhibition of CHE in higher concentrations of the two components of the mixture and no influence on LDH activity. We determined that the model which best describes biomarkers activity for these enzymes are concentration

addition. Our results show that there is a synergism between ametryn and diuron, which enhances its effects on Danio rerio. Risk assessment which uses data from the single substances may underestimate the real risk of these substances, as it is almost impossible to found them isolated in nature.

ET18-2

Baseline levels and trophic transfer of persistent organic pollutants in sediments and biota from the Congo River Basin (Democratic Republic of Congo)

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The present study aims to evaluate the occurrence of persistent organic pollutants (POPs) in the Congo River Basin (CRB) and to investigate the trophic transfer of POPs through a tropical freshwater aquatic food web using stable isotopes. To our knowledge, no data on levels of POPs in sediment and biota from the CRB are present in the literature. In addition, studies on biomagnification profiles of POPs using δ13C and δ15N are scarce in tropic regions.
Samples of sediment and biota (i.e. invertebrate and fish species) were collected from three tributaries (Lomami, Aruwimi and Itimbiri) and the Congo River itself to determine the degree of contamination by POPs (PCBs, PBDEs, DDTs, HCHs, CHLs and HCB) with a GC-MS. Stable isotope (δ13C and δ15N) measurements in plant, invertebrate and fish species were performed using an EA-IRMS.
POP levels in the sediment and biota are low compared to results of other studies from temperate and tropical regions, with exception of total PCB levels found in fish from the Itimbiri River (average ΣPCB = 1465±920 ng/g lipid weight lw). The PCB levels are of the same magnitude as found in industrialised basins. This might be related to the presence of industrial and agricultural (palm oil) activity in the Itimbiri River Basin. Of the POPs analysed, total PCBs were the predominant contaminants with concentrations ranging from 21 to 3664 ng/g lw, followed by DDT and its metabolites at 4 to 504 ng/g lw, PBDEs at 2 to 188 ng/g lw and HCHs at 2 to 66 ng/g lw.
The values of δ13C and δ15N in biota ranged from -40.5" to -22.6" and from -2.3" to 16.5", respectively. On average, δ15N increased from primary producers to invertebrates to fish. The understanding of the trophic transfer of environmental contaminants in biota of the CRB is critical to evaluate the influence of these contaminants on ecosystems and human life. The observed increase in δ15N with increasing PCB 153 concentrations indicates that trophic levels play an important role in the movement of contaminants through the Itimbiri food web (R2= 0.66, p=0.0012). The slope of the regression equation (0.35) is an index for biomagnification. These correlations are currently determined for all individual contaminants. Slopes will be compared with results from other studies around the world and will be used for risk assessment.

ET18-3

Avian risk assessment for the Neotropics: methods to find appropriate species

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In the context of pesticide registration a lot of emphasis has been given to European farmland birds during the last decade. In Europe the concept of focal species has been implemented by the EU authorities and a wealth of data has been gathered. We conducted several field studies in the neotropics to evaluate the usefulness of different survey methods given the local conditions of high species diversity, different behavioural traits and different taxonomic affiliations of the birds and to gain some preliminary insight into bird communities that are found on neotropical agricultural land.

ET18-4

First evidence of linked physiological and demographic effects of chlordecone in the freshwater shrimp Macrobrachium faustinum (Decapoda, Palaemonidae) in the Pérou River in Guadeloupe (FWI)

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From 1972 to 1993, the organochlorine insecticide chlordecone was intensively used as Kepone and Curlone against the banana root borer in the French West Indies. Chlordecone is resistant to degradation in the environment, and it is adsorbed for hundreds of years in soils of Caribbean islands where it was used. Driven by water cycle, it steadily gets transferred to aquatic ecosystems. Chlordecone has a high potential for bioaccumulation in freshwater fish and shrimps. From 2009 to 2010, a field study was undertaken to characterize the physiological and demographic effect induced by the bioaccumulation of chlordecone in the freshwater shrimp Macrobrachium faustinum. Individuals were sampled at 7 dates upstream and downstream of the Pérou River located southeast of the Basse-Terre island of Guadeloupe (FWI). Tissular chlordecone concentration and biochemical parameters specifically related to the mode of action of the molecule (Glutathione S-transferase and Na+/K+-ATPase activities; 20-hydroxyecdysone and vitellogenin levels) were simultaneously measured in the organisms, so that a direct relation between bioaccumulation and physiological alterations can be established. In parallel, body length of the individuals was used to follow size cohorts and to study the dynamics of the population. The results show a correlation between water concentration in chlordecone and the amount of compound accumulated in the tissue of M. faustinum. Water and abdomen tissue concentrations were higher downstream, as compared to upstream, with concentration factors by M. faustinum of 2600 and 1000, respectively. Glutathione S-transferase activity was inhibited in all the individuals whereas no change was observed for Na+/K+-ATPase. The level of 20-hydroxyecdysone was higher in the females sampled downstream, and vitellogenin was detected in all the males found in the samples. Population dynamics analysis showed that chlordecone did not increase mortality but it affected individual growth. Moreover, population modelling showed that whatever the season, the maximal mean individual size (L) was lower downstream as compared to upstream. Slower growth of the individuals sampled downstream may result from high 20-hydroxyecdysone levels measured in those individuals. For the first time, it has been indicated that bioaccumulation of chlordecone by a freshwater shrimp resulted in physiological alterations that might affect population dynamics.

ET18-5

Environmental risk assessment of pesticides used in sugarcane plantations in the Central Amazon - Brazil

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The sugarcane production has registered a significant increase in Brazil during the last years mainly due to the increasing demand of biofuels. The Amazon region has the highest indices of sugarcane production expansion in the country. Sugarcane is the fourth crop in consumption of pesticides in Brazil, relying on a wide variety of herbicides and insecticides. Pesticides used in sugarcane plantations of the Amazon are likely to reach down-stream aquatic ecosystems that are considered to be hot-spots of species diversity. In this study, pesticide risks for freshwater ecosystems surronding sugarcane plantations were assessed considering pesticide spray-drift and runoff as the main source of pesticide entry into aquatic ecosystems. The PRIMET model was used to calculate Predicted Environmental Concentrations (PECs) of pesticides produced by spray-drift deposition. Pesticide runoff inputs were calculate using the Reus runoff formula. Risks were calculated using Toxicity Exposure Ratios (TERs), calculated by dividing PECs by Predicted No Effect Concentrations (PNECs). PNECs were derived using toxicity data for standard aquatic test organisms. For the pesticides that showed risk, a refined risk assessment was performed using semi-field toxicity data with the PERPEST model. A clear risk was estimated for 3 pesticides, 2,4-D amine, diuron and metribuzin, out of the list of the 14 pesticides evaluated. Probabilities to find clear effects higher than 25% were estimated for fish and tadpoles, macrocrustaceans and macrophytes for 2,4-D amine. Molluscs were the only taxonomic group that presented no risks for diuron, and all the studied endpoints showed probabilities to find clear effects higher than 25% for metribuzin. The results of this study suggest that pesticides used in sugarcane plantation in the Amazon are posing risks for aquatic biodiversity in surrounding ecosystems, showing that the model of biofuels production that is currently established in the Amazonian region can be environmentally unsustainable due to the intense pesticide applications.

ET18-6

Is the risk of pesticides in tropical countries different from that in other parts of the world?

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Literature surveys show that pesticides tend to dissipate faster in tropical countries than in temperate regions. This fact has led to the assumption that ecological effects and environmental risks of pesticides in tropical countries are reduced, since exposure times in such countries are considerably shorter than in temperate regions of the world. Given that the toxicity of most organic compounds to tropical species is practically no different from their toxicity to non-tropical species, the question remains whether the actual risk of pesticides is less under tropical conditions. To elucidate this query, a comparison of environmental risks of pesticides between tropical and non-tropical regions was carried out, using experimental data from the literature and fugacity modelling. Tropical field data included dissipation and sorption characteristics of 73 pesticides, which were compared to non-tropical data for the same compounds obtained from databases. Exposure modelling was based on the physico-chemical properties of the pesticides, and outputs were obtained for the two scenarios to be compared, i.e. tropical and non-tropical conditions. Results from modelling show that except for a few cases, the level of risk of exposure for most pesticides in tropical agriculture is similar to that in other climatic regions of the world. Generally, dissipation of pesticides increases under the warm and wet conditions of the tropics, with most of the dissipation occurring through hydrolysis in water and biological degradation in water and soil.

High temperatures in the tropics also foster volatilisation rates, while high precipitation and poor soils tend to increase losses into runoff and, for certain chemicals, their leaching behaviour. The environmental risk appears to be determined by a balance between soil types, soil organic carbon, pH and the rates of degradation in the various environmental compartments. Therefore, the actual risk of pesticides depends more on the specific soil types and water properties of the areas concerned than on climatic features of the tropical regions.

ET19A - Veterinary medicines in the environment: basic research for risk analysis

ET19A-1

Dissipation and sequestration of veterinary antibiotics in soil under variable environmental conditions

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Knowing the impact of environmental conditions on the fate of veterinary medicines in soil is crucial for a thorough understanding and modelling of their environmental fate, especially when transferring laboratory results to the field situation. Particularly soil temperature and moisture are known to affect the dissipation and sequestration of organic contaminants in soil. To study the impact of these parameters on the dissipation and sequestration of veterinary antibiotics, we conducted laboratory experiments with varying soil temperature and moisture. Furthermore, we conducted a field experiment to validate our results under realistic conditions. All experiments were performed with two compounds of contrasting sorption behavior, the weakly sorbing sulfonamide antibiotic sulfadiazine (SDZ) and the strongly sorbing fluoroquinolone antibiotic difloxacin (DIF). Bioaccessibility and sequestration of the compounds in soil were characterised by sequential extractions of increasing harshness.
In laboratory experiments, the dissipation of SDZ proved to be accelerated by a factor of two to three with a temperature increase of 11°C. We successfully used the temperature-dependence of dissipation rate constants that was derived from these laboratory experiments to predict temporal changes of SDZ concentrations in the field experiment. When the soil moisture was either very high or low, however, systematic deviations between measured and predicted concentrations were observed, particularly for the sequestered antibiotic fraction. We conclude that temperature is the main factor controlling the dissipation rates of SDZ. Sequestration is additionally affected by soil moisture, presumably due to conformational changes of soil organic matter with wetting and drying of the soil. Experiments with drying-wetting cycles in climate chambers are currently conducted to further clarify the effects of soil moisture on the dissipation and sequestration of SDZ. The results of these experiments will be presented. Soil temperature and moisture surprisingly had no impact on the dissipation of DIF. The residual concentrations of this compound remained on the same constant level across all experiments. In contrast to the weakly sorbing SDZ, the strong sorption of DIF presumably limits the impact of environmental conditions on its fate. We conclude that the fate of strongly sorbing pharmaceuticals is less dependent on environmental conditions than the fate of weakly sorbing compounds.

ET19A-2

Fate, metabolism and uptake plant uptake of selected veterinary drugs in the rhizosphere of maize

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Root-soil interactions in the rhizosphere include various processes influencing the soil solution chemistry. A rhizosphere box system is well suited to demonstrate the influence of the root on the fate and metabolism of antibiotics. By the help of this micro ecosystem it is possible to measure the metabolite patterns in the phase and in rhizosphere pore water without further sample clean up and at high spatial and temporal resolution. In addition the usage of 14C-labelled compounds will reveal the mass balance of the applied drug including formation of NER (non-extractable residues) and mineralization.
The influence of the maize rhizosphere on the fate and behaviour of two veterinary antibiotics sulfadiazine (SDZ) and difloxacin (DIF) and their metabolites in soil was investigated in suitable microcosms. Radioactivity was traced and LC-MS/MS analyses of pore water and soil was performed. The pore water analysis of soil at various distances from the rhizosphere of maize plants revealed no influence on the fate of the applied compounds. After incubation for 55 days with SDZ the soil analysis at various distances from the roots of the plant revealed the considerable impact of this biological hotspot. The mineralization rate near the roots was 10 times higher than for bulk soil. Furthermore the results revealed that up to 70% of the applied compounds in the root area were degraded to unknown metabolites. In order to correlate chemical and biological data, results on the abundance and transferability of bacterial antibiotic resistance genes were analyzed in parallel and confirmed that the effects were most pronounced under conditions of maximum SDZ transformation. Development of a new analytical method to detect plant uptake of the applied drugs yielded results revealing only trace amounts (maximum of 1% of the applied compounds) mainly in the roots (99%) of the maize plants. The experimental setup using the fluoroquinolone DIF revealed only a minor impact of the rhizosphere on the fate of the drug applied. The mineralization rate (<0.5%) and the plant uptake (< 0.5%) were negligible over the whole experimental period. The radioactive balance revealed that 99% of the applied radioactivity was still detectable in soil.

ET19A-3

Fate of sulfadiazine and its effects on abundance and transferability of antibiotic resistance in rhizosphere and bulk soil

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Veterinary antibiotics reaching agricultural lands with manure pose the risk of spreading of antibiotic resistance. The rhizosphere is a hot spot for horizontal gene transfer but might also facilitate the degradation of antibiotics. On the one hand, it was shown that the addition of artificial root exudates increased the bacterial community tolerance towards the antibiotic compound sulfadiazine (SDZ), indicating that the rhizosphere might be a hotspot of resistant bacteria. On the other hand, the exposure of bacteria to SDZ is presumably reduced in the rhizosphere since the dissipation of bioaccessible SDZ-concentrations was recently shown to be accelerated in rhizosphere soil. However, little is known about the abundance and dynamics of sulfonamide resistance genes in the rhizosphere. We therefore compared the fate and effect of SDZ in bulk- and rhizosphere soil in mesocosms planted with maize and in field plots planted with maize or grass. Both were treated with manure from pigs which either received SDZ or not. A sequential SDZ-extraction protocol for soil yielded antibiotic fractions of increasing binding strength serving as a proxy for the bioaccessible concentration. Following the application of manure, easily extractable concentrations of SDZ and its metabolites decreased rapidly in bulk soil whereas residual concentrations decreased much slower. In rhizosphere soil, easily extractable and residual concentrations of SDZ were mostly lower than in bulk soil indicating an accelerated degradation. Quantitative real-time PCR detection in total community DNA showed that the application of manure containing SDZ increased the relative abundance of the SDZ resistance genes sul1 and sul2 in bulk- and rhizosphere soil of maize, which may be linked to the increased abundance of LowGC-type plasmids. In the field experiment, this increase was prolonged only in the rhizosphere of grass and maize even at bioaccessible SDZ-concentrations below previously reported effective doses. Likely, the continuous selection of resistant bacteria in the rhizosphere was fostered by a higher microbial activity together with a constant but low exposure to SDZ.

ET19A-4

Degradation of sulfadiazine in lysimeters, soil microcosms, and pure cultures

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Among sulphonamides, sulfadiazine (SDZ) is one of the most widely applied veterinary antibiotics. Long-term lysimeter studies with manure from pigs treated with 14C-labelled sulfadiazine (SDZ) gave the first hint that considerable amounts of the radiolabelled SDZ applied to the soil could have been mineralized within 3 years, although, up to now, mineralization of SDZ in soils was supposed to play only a minor role. Three years after the application of the 14C-manure, 50% of the applied radioactivity was found in the non-extractable fraction (NER) of the lysimeters and the readily extractable fraction (RES) was about 10%. Small amounts of the radioactivity were recovered in 30 to 100 cm depth and 0.5% in the percolate. Overall 65% of the applied radioactivity was recovered, meaning that 35% failed in the balance. Microcosm experiments showed a mineralisation of up to 10% of the added SDZ in three months, when incubated at 45% of the WHCmax and up to 50-60%, when incubated as slurry (one part soil, 4 parts water). Thus we found strong evidence, that mineralisation of SDZ could have been the reason for the loss of radioactivity in the lysimeter experiment and that water content plays a crucial role. Furthermore, we enriched mixed and isolated pure bacterial cultures which were capable of SDZ degradation in absence of soil matrix. Extensive analysis by radio-HPLC, LC-MS/MS and FT-ICR-MS/MS revealed more details about the fate of SDZ in our cultures. Disappearance of SDZ always went along with appearance of a principal metabolite identified as 2-amino-pyrimidine. 2-amino-pyrimidine was already quoted in the literature as a photolysis product. In our experiments, 2-amino-pyrimidine originated definitely from microbial activities.

ET19A-5

Impacts of a decade of annual tylosin, chlortetracycline and sulfamethazine application on drug persistence, bacterial phylogenetic diversity and functional diversity in an agricultural soil

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